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Efectes de l'associació de varietats de blat sobre l'abundància d'espècies arvenses, de pugons i la producció en cultius ecològics

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Memòria presentada per Alba Tous Fandos per optar al grau de doctora per la Universitat de Barcelona

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Alba Tous Fandos

El director i tutor de la tesi

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Agraïments

Com tot en aquesta vida, aquesta tesi no hagués set possible sense un conjunt de persones que directe o indirectament han contribuït en la seva creació. Si hagués d'agrair a totes elles ben segur que acabaria duplicant les pàgines de la tesi. Així doncs, em limitaré als companys de la Universitat i aquelles persones més properes.

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Des de l'inici del doctorat he tingut clar que el ritme de la tesi, el creixement com a investigadora i el nivell d'estrès varia completament depenen d'on treballis. Jo he tingut la sort de caure en un bon grup, el d'agroecologia de la Universitat de Barcelona, on tot i el nivell de feina general, m'he sentit acompanyada i guiada durant el procés.

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Abstract

Organic arable cropping systems encourage crop diversification to reduce the use of external inputs and attend to agroecosystem simplification. The combination of different species (interspecific polyculture) or cultivars of the same species (intraspecific polyculture) can result in a series of agroecosystem services, such as weed regulation, aphid control and improved yield. The advantages of polycultures rely on the capacity of the different species or cultivar to provide different functions into the agroecosystem. These functions can mean to be a good crop competitor against weeds, or to develop pest resistance.

The doctoral thesis aims to delve into the effects of wheat cultivar mixture on weed and aphid control and improving yield through four complementary studies. The studied wheat cultivars are the modern cultivar Florence-Aurora and the traditional Xeixa, Montcada (*Triticum aestivum* L. subsp. *aestivum*), and Forment (*Triticum turgidum* L. subsp. *durum*). These cultivars were selected based on functional traits, such as height, phenology, and aphid resistance, as well as bread-making qualities such as gluten strength and aroma.

The first two studies were field experiments conducted in the Espai d'Interès Natural of Gallecs, a periurban agricultural area near Barcelona. The first experiment evaluated the influence of soil management practices (tillage and fertilization) on pest control, weed regulation, and yield of Florence-Aurora, Xeixa, and Forment monocultures, as well as the resulting mixture.

The second experiment analyzed aphid population control and yield of Florence-Aurora, Montcada, and Forment monocultures, the association of Florence-Aurora and Montcada (cultivars with similar traits), Florence-Aurora and Forment (cultivars with different traits), and the incorporation of burclover (*Medicago polymorpha)* undersowing. This study also explores bottom-up and top-down mechanisms on aphid control through the analysis of the abundance and richness of predatory arthropods and parasitism rates.

The third and fourth studies were conducted under controlled greenhouse and laboratory conditions, focusing on the associative resistance of wheat cultivar mixtures at different stages of aphid host selection and population development. These experiments allowed for the evaluation of three functional traits, tissue nitrogen content, above-ground biomass and odor profile, as well as the influence of plant-plant and plant-aphid interactions on these traits.

The results indicate that Florence-Aurora is highly susceptible to aphids and weed infestation. Associating Florence-Aurora with taller cultivars, Forment and Xeixa, favors weed regulation because it increases the competition against weeds.

Mixing cultivars with different functional traits, Florence-Aurora with Forment, and the incorporation of an undersowing in Florence-Aurora monoculture, benefits aphid control through bottom-up mechanisms since it does not affect predator abundance or parasitism rates. In contrast, mixing cultivars with similar traits, Florence-Aurora with Montcada, and the association of Florence-Aurora with Forment and the legume cover crop, do not contribute to pest control because of functional redundancy among the combined organisms.

Wheat crops showed similar production. Experiments under natural conditions indicate that tillage practices alter weed and aphid infestations without affecting crop functionality. However, the production of Florence-Aurora monoculture and the cultivar mixture was negatively affected by non-fertilization and chisel ploughing conditions.

Finally, laboratory experiments demonstrated that aphid control in cultivar mixtures depends on the identity and interaction of the combined wheat cultivars. The association of cultivars with different odor profiles, Florence-Aurora with Forment, altered the odor of the mixture. This modification improved the mixture's resistance at the host localization phase, as aphids are less attracted to the odor signals of the mixture than to those of the constituent monocultures. Moreover, Florence-Aurora and Forment differed in tissue nitrogen content and their combination resulted in a decrease in Florence-Aurora’s nitrogen content. Nevertheless, no relationship was found between wheat nitrogen content and aphid abundance.

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Introducció general

1. La simplificació dels sistemes agrícoles

Els ecosistemes agrícoles han estat modificats al llarg del temps per la producció d'aliments, fibres, farratges i, més recentment, bio-combustibles. És a dir, són sistemes antropogènics vinculats a les activitats humanes, als contextos socioeconòmics i als avenços tecnològics (Di Bene et al. 2022). En aquest sentit, a mitjans del segle XX, l'agricultura va experimentar una forta intensificació, l'anomenada "Revolució Verda" amb la finalitat d'augmentar la producció d'aliments per fer front a les necessitats d'una població constantment creixent (Tilman et al. 2002).

La intensificació agrícola en els cultius herbacis extensius ha implicat principalment un gran augment de la fertilització nitrogenada i de fòsfor, principalment d'origen químic, l'ús elevat de pesticides, la reducció o abandonament de les rotacions de cultius, i la llaurada intensiva (Tilman et al. 2002). A més, l'expansió dels monocultius de cereals i la substitució de la sembra de varietats tradicionals per la sembra d'unes poques varietats comercials modernes ha provocat una pèrdua de la diversitat conreada i una forta simplificació dels sistemes agrícoles (Sans et al. 2013; Ficiciyan et al. 2018; Dainese et al. 2019).

Les varietats modernes s'han originat a partir d'encreuaments genètics (o altres tècniques de millora varietal) amb la finalitat d'augmentar la producció. Tanmateix, són varietats més susceptibles a plagues i malalties i, per tant, requereixen aportacions externes de fitosanitaris tant pel control de plagues com per a regular les poblacions d'espècies arvenses (Newton et al. 2010; Mefleh 2021). En canvi, les varietats tradicionals són més resistents i presenten més resiliència, a més de destacar per l'alt valor nutricional i cultural (Newton et al. 2010; Ficiciyan et al. 2018).

2. El paper de la diversitat funcional en els sistemes agrícoles

Per tal de reduir la dependència de les aportacions externes, l'agricultura ecològica promou la diversitat agrícola i les interaccions ecològiques entre organismes d'igual o diferent nivell tròfic (IFOAM 2008, Rodriguez et al. 2021). La diversitat agrícola s'entén com tots aquells organismes que coexisteixen i interaccionen en l'ecosistema agrícola. Aquesta inclou la diversitat planificada, que fa referència a aquella que els pagesos incorporen a la parcel·la (el cultiu pròpiament), i la diversitat associada, que aplega tots els organismes presents de manera natural en el camp, des dels microorganismes fins a la flora i fauna. Dins de la diversitat planificada es pot diferenciar entre la diversitat genètica, com ara l'associació de varietats d'una mateixa espècie, i la diversitat específica, que engloba la rotació, l'associació de cultius, la incorporació dels cultius de cobertura i les bandes florals (Andow 1991) (Figura 1). La gestió de la diversitat planificada afecta a la diversitat associada; així, per exemple el manteniment de bandes florals pot afavorir la presència de fauna auxiliar beneficiosa com predadors o pol·linitzadors que provenen dels hàbitats adjacents (Altieri & Rogé 2009).

El conjunt de la diversitat d'organismes i les seves interaccions s'anomena complexitat. En els inicis de l'agricultura ecològica, s'apostava per sistemes agrícoles complexos per tal d'obtenir una sèrie de beneficis entre els quals destacava el control de plagues (Altieri & Rogé 2009; Dainese et al. 2019). Aquests beneficis posteriorment es van anomenar serveis agroecosistèmics. Els serveis agroecosistèmics es classifiquen en serveis d'aprovisionament, com ara la producció de gra; serveis de regulació, com serien el control de plagues, el control de les poblacions d'espècies arvenses i la pol·linització; serveis culturals, per exemple la recuperació de varietats tradicionals; i finalment en serveis de suport, com és el cas de la fixació biològica del nitrogen (MEA 2005). Tanmateix, l'establiment de sistemes complexos no sempre es tradueix en la provisió de serveis, ja que, les interaccions que s'estableixen entre les varietats i espècies associades poden ser negatives com la competència pels recursos, i perjudicar el correcte desenvolupament del cultiu (Barbosa et al. 2009; Gaba et al. 2014; Barot et al. 2017).

Figura 1 Associacions de varietats de blat (diversitat planificada) en els experiments en condicions naturals a l'Espai d'Interès Natural de Gallecs (Mollet del Vallès, Barcelona). a. Policultiu de tres varietats de blat (*Triticum* sp.); D'esquerra a dreta, Florence-Aurora, Forment i Xeixa (maig 2020), b. Associació de blat amb una coberta de melgó (*Medicago polymorpha*) (maig 2020), c. Barreja de dues varietats de blat, Florence-Aurora i Forment, associada amb una coberta de melgó (juny 2020). Fotografies d'Alejandro Pérez Ferrer (a), Alba Tous Fandos (b) i Lourdes Chamorro Lorenzo (c).

La diversitat funcional va ser definida per Tilman (2001) com aquells components de la diversitat que contribueixen a les interaccions beneficioses i que aporten resistència, ús eficient dels recursos, estabilitat i resiliència al sistema agrícola. El disseny de policultius (associacions de cultius o de varietats) funcionals en sistemes herbacis ha de tenir en compte els següents aspectes principals:

Es requereix la selecció d'espècies i varietats adaptades al context agronòmic, edàfic i climàtic local. Les varietats tradicionals acostumen a respondre millor a les condicions ambientals de cada regió (Serra-Gironella & Álvaro 2017; Altieri & Rogé 2009).

Cada espècie o varietat disposa d'un conjunt de trets funcionals específics (característiques morfològiques, ecofisiològiques i fenològiques) que poden influir en la funcionalitat del cultiu i en conseqüència afectar la provisió de serveis agroecosistèmics (Lavorel & Garnier 2002; Gaba et al. 2015). De manera general, és important associar espècies o varietats amb trets funcionals complementaris (Barot et al. 2017; Brooker et al. 2021). Per exemple, les varietats de blat de port alt (tret funcional) són més eficients en l'aprofitament de la llum (funcionalitat ecosistèmica) i això pot atorgar al cultiu un avantatge competitiu sobre les poblacions d'espècies arvenses (servei agroecosistèmic de regulació) (Worthington et al. 2013) (Figura 2).

Al mateix temps, s'han d'afavorir les interaccions positives, com ara la facilitació, complementarietat o compensació, entre les espècies o varietats associades (Barbosa et al. 2009; Brooker et al. 2021). En aquesta línia, l'ús d'una coberta vegetal de lleguminosa és una pràctica freqüent en els cultius de cereal per la interacció de facilitació entre la lleguminosa i el cereal. La lleguminosa aporta nitrogen al sòl gràcies a la capacitat de fixar el nitrogen atmosfèric i aquesta provisió de nitrogen beneficia al desenvolupament del cereal (Wezel et al. 2014).

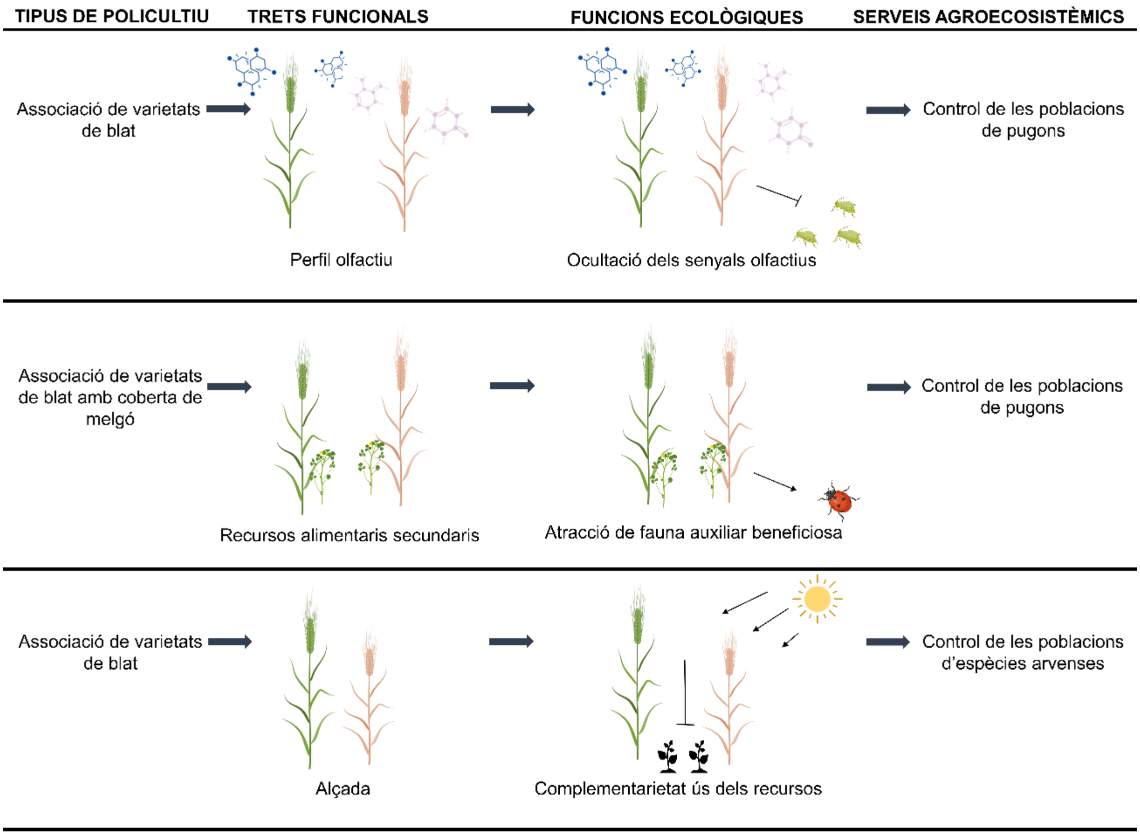


Figura 2 Esquema que il·lustra la relació entre el tipus de policultiu (associació de varietats de blat i la incorporació d'una coberta de melgó), els trets funcionals oferts per les varietats i les espècies combinades, les funcions ecològiques associades als trets i finalment el servei agroecosistèmic que en deriva.

3. El valor de l'associació de varietats de blat en la provisió de serveis agroecosistèmics en cultius extensius de cereals

El blat d'hivern (*Triticum aestivum* L.) és un dels conreus majoritaris a escala global i local. A Catalunya, el cultiu extensiu de cereals de secà representa el 40,92% de les terres cultivables i els cultius principals són el blat d'hivern i l'ordi (IDESCAT 2022). Així mateix, la barreja de varietats de cereal, l'anomenat mestall, ha estat una pràctica usada a Europa des del segle XVIII per afavorir la producció. Els darrers anys, el seu ús ha anat a l'alça en diversos països europeus, tot i que encara és poc freqüent a Catalunya (Costanzo & Bàrberi 2014; Wezel et al. 2014; Borg et al. 2018)

El valor potencial de les associacions de varietat de blat rau en l'enorme heterogeneïtat genètica entre varietats (Barot et al. 2017). L'adequat assemblatge d'aquesta variabilitat de trets funcionals únics i diferenciadors poden optimitzar l'ús de recursos del sistema agrícola i, per tant, millorar la funcionalitat dels cultius i, al seu torn, la prestació de serveis agroecosistèmics (Costanzo & Bàrberi 2014; Tilman et al. 2014; Reiss & Drinkwater 2018)

Entre els beneficis de l'associació de varietats de cereals destaquen la provisió de serveis de suport com l'eficiència en l'ús dels nutrients (Yan et al. 2018), serveis de regulació com la competència davant de poblacions d'espècies arvenses (Kiær et al. 2009), la resistència a patògens (Gibson & Nguyen 2021) o bé, als estressos abiòtics (Costanzo & Bàrberi 2014), i serveis d'aprovisionament com la producció de gra (Reiss & Drinkwater 2018).

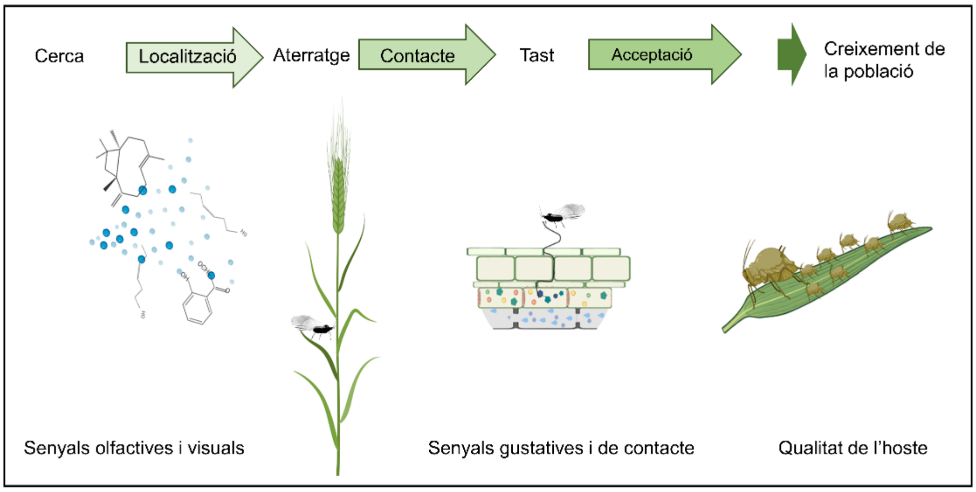
En els següents subapartats, s'exposa els beneficis dels policultius de blat en el control de les poblacions pugons, en la regulació de les poblacions d'espècies arvenses i en la producció de gra, ja que són els principals aspectes estudiats en la tesi doctoral.

3.1. Control de les poblacions de pugons

Els pugons són insectes herbívors fitòfags que es consideren plagues potencials en els cultius de cereal, pel seu impacte en la producció. En els cultius de blat, els pugons redueixen la producció de manera directa perquè succionen els nutrients de la planta hoste i de manera indirecta perquè actuen de vector de transmissió de virosis com la del nanisme i de l'esgrogueïment (BYDV, Barley Yellow Dwarf Virus), entre d'altres (Dedryver et al. 2010). A més, els pugons han estat àmpliament utilitzats com a organisme model en els estudis de la relació planta-fitòfag (Rodriguez-Saona & Stelinski 2009).

El procés de selecció de l'hoste i el posterior desenvolupament de la població de pugons és complex i compren diferents fases (Powell et al. 2006) (Figura 3). La primera fase és la cerca de la planta hoste, on els pugons a partir d'estímuls olfactius i, en menor mesura, visuals localitzen el seu hoste. El perfil olfactiu és el conjunt de compostos volàtils que emet constantment una planta i serveix per a la comunicació planta-planta. També conté la informació sobre la identitat i la qualitat de la planta emissora. Els pugons presenten un òrgan olfactiu anomenat estilet, que capta de forma precisa aquests senyals olfactius i usen la informació per seleccionar l'hoste (Webster 2012).

La segona fase és la d'acceptació, on els pugons avaluen la qualitat de l'hoste mitjançant estímuls olfactius, tàctils i gustatius. Finalment, si accepten l'hoste, es desenvolupa la colònia de pugons. El creixement de la colònia i la mida dels pugons dependrà de la qualitat de l'hoste (Aqueel & Leather 2011; Nowak & Komor 2010).



Procés de selecció de l'hoste i desenvolupament de la població de pugons

Figura 3 Esquema del procés de selecció de l'hoste i el desenvolupament de la població de pugons. Il·lustració inspirada en l'estudi de Powell et al. (2006).

El control de plagues en associacions de varietats de blat està relacionat amb dos conjunts -no exclusius- de processos ecològics. Si la regulació de la població de pugons recau en les espècies o varietats resistents, es tracta de mecanismes *bottom-up*. En canvi, si la regulació prové de nivells tròfics superiors, com ara els artròpodes predadors (coccinèl·lids, aràcnids, etc.) i vespes parasitoides, s'anomenen mecanismes *top-down* (Barbosa et al. 2009; Gurr et al. 2017).

Els mecanismes de control *bottom-up* es vinculen directament amb la diversitat planificada i les relacions de facilitació entre les varietats resistents i susceptibles a la infestació. Aquestes interaccions positives poden provocar modificacions morfològiques o fisiològiques que incrementen la resistència de les varietats més susceptibles (Barbosa et al. 2009; Ninkovic et al. 2016; Dahlin et al. 2020). A més, les varietats més sensibles als pugons són ocultades, tant visualment com per senyals olfactius, per les varietats més resistents, i d'aquesta manera en dificulta la localització (Tahvanainen & Root 1972; Finch & Collier 2000). El conjunt d'aquests mecanismes *bottom-up* confereixen al cultiu l'anomenada resistència associativa (Barbosa et al. 2009).

Per tal d'afavorir la resistència associativa és important estudiar els trets funcionals que ofereixen resistència als pugons, la complementarietat entre aquests atributs i si les interaccions entre les varietats de blat poden afectar la funcionalitat d'aquests trets (Lithourgidis et al. 2011; Gaba et al. 2015; Brooker et al. 2021). Tres dels principals trets funcionals relacionats amb el control de la població de pugons són el perfil olfactiu, el contingut de nitrogen tissular i la biomassa aèria. Mentre el perfil olfactiu de les varietats de blat té un paper directe en la fase de localització i selecció de l'hoste (Pickett et al. 1992; Webster 2012), el contingut de nitrogen en el teixit vegetal i la biomassa aèria estan relacionats amb la qualitat de l'hoste i, en conseqüència amb la taxa de creixement de les poblacions de pugons (Aqueel & Leather 2010; Nowak & Komor 2010).

El contingut de nitrogen del teixit vegetal és un factor limitant en la dieta dels pugons, ja que el seu contingut en el floema és escàs (~ 0,1 N:C) (Taiz & Zeiger 2006). Estudis experimentals realitzats en condicions d'hivernacle han constatat una relació positiva entre la mida, la longevitat i la fecunditat dels pugons i la intensitat de la fertilització del cultiu amb nitrogen (Duffield et al. 1997; Aqueel & Leather 2011; Nowak & Komor 2010).

Els mecanismes de control *top-down* es fonamenten en el fet que la diversitat agrícola genera les condicions adequades per atreure la fauna auxiliar beneficiosa com són els artròpodes depredadors i parasitoides dels pugons (Koricheva & Hayes 2018). Entre d'altres, la diversitat planificada ofereix la provisió de recursos alimentaris per la fauna útil com ara llocs per fer postes o refugiar-se i hostes alternatius (Gurr et al. 2017).

3.2. Control de la població d'espècies arvenses

Un dels principals reptes que té l'agricultura ecològica en els cultius herbacis és la gestió de les poblacions d'espècies arvenses, ja que està prohibida l'aplicació d'herbicides i el control mecànic de vegades no és suficient per reduir les poblacions per sota del llindar de competència. Encara hi ha pocs estudis que hagin analitzat l'efecte de l'associació de varietats de cereals sobre el control de les espècies arvenses. No obstant això, diversos treballs suggereixen que la combinació de varietats que difereixen en l'alçada, la biomassa aèria i el nombre de fillols per planta podrien augmentar la capacitat competitiva enfront de les poblacions arvenses a causa d'ocupar de manera més eficient l'espai aeri i els recursos lumínics, i d'absorbir els nutrients del sòl (Worthington et al. 2013; Lazzaro et al. 2018).

3.3. Augment de la producció de gra

Els serveis agroecosistèmics d'aprovisionament, com l'increment de la producció del cultiu i la seva estabilitat en el temps, són els serveis més apreciats pels productors a l'hora de valorar la incorporació de la diversitat planificada als seus camps (Di Bene et al. 2022).

Diversos estudis han assenyalat que l'associació de varietats de cereals modernes amb varietats tradicionals confereixen al policultiu més resiliència davant les condicions ambientals adverses i conseqüentment, una major estabilitat en la producció (Reiss & Drinkwater 2018; Ficiciyan et al. 2018; Serra-Gironella 2020). L'estudi metanalític de Borg et al. (2018) mostra que la producció de l'associació de varietats de blat d'hivern és entre el 4% i el 6% més gran que la dels respectius monocultius.

A més, la combinació de varietats amb diferents trets biològics com l'alçada, la biomassa aèria i el desenvolupament fenològic, pot afavorir les interaccions complementàries que atorguin un millor aprofitament de la llum i dels recursos nutritius del sòl i, en conseqüència, una millora de la producció del cultiu (Worthington et al. 2013; Lazzaro et al. 2018).

Justificació de la tesi doctoral

La major part de la producció ecològica mundial de blat prové de conreus de varietats modernes en règim de monocultiu. La sembra de varietats tradicionals és poc comuna per la baixa disponibilitat en el mercat i perquè l'agricultura ecològica copia alguns aspectes dels models agronòmics convencionals intensius, on les varietats tradicionals resulten menys productives. A més, la implementació de policultius és limitada, per una banda, pel sobreesforç logístic i econòmic que genera el cultiu simultani de varietats i espècies diferents (Lin 2011; Di Bene et al. 2022). Per l'altra banda, perquè l'associació de varietats d'una mateixa espècie és una pràctica poc estesa a Europa, tot i suposar una gestió agrícola i una inversió econòmica similars als monocultius (Vanloqueren & Baret 2009). Tanmateix, en els darrers anys, ha crescut l'interès per part d'investigadors i agricultors per l'ús de policultius i la sembra de varietats tradicionals en conreus herbacis extensius ecològics amb la finalitat d'incrementar la complexitat i la salut global del sistema i de reduir la dependència a les aportacions externes (Wezel et al. 2014; Reiss & Drinkwater 2018; Di Bene et al. 2022).

La tesi doctoral pretén avançar en el coneixement dels beneficis i perjudicis de la diversificació dels cultius sobre la regulació de les poblacions arvenses, el control de pugons i la producció de gra en conreus de blat mitjançant estudis en condicions naturals i controlades d'hivernacle i laboratori. Gran part de la recerca en aquest àmbit s'ha dut a terme mitjançant experiències en condicions controlades de laboratori o hivernacle. En aquests estudis, no es valora la influència dels factors ambientals o agronòmics sobre la funcionalitat dels policultius ni l'efecte de la diversitat planificada sobre la diversitat associada. A més, pocs experiments consideren els trets funcionals a l'hora de dissenyar els policultius, fet que pot propiciar l'establiment de policultius poc avantatjosos a causa de redundàncies o interaccions negatives entre les varietats associades. Finalment, els estudis que avaluen els trets funcionals de distintes varietats o espècies, acostumen a caracteritzar els trets en individus crescuts en règim de monocultiu. Així doncs, no es té en compte la possible modificació dels trets funcionals fruit de la interacció planta-planta present en els policultius (Gaba et al. 2015; Barot et al. 2017; Brooker et al. 2021).

Amb la finalitat d'aprofundir en el paper de les varietats de blat associades sobre el funcionament dels policultius en condiciones naturals, s'ha dissenyat un experiment de dos anys amb l'associació de tres varietats de blat, una relativament moderna i dues de tradicionals i, un altre estudi també de dos anys de duració, amb dues associacions cadascuna d'elles formada per una varietat moderna i una de tradicional, a més de la incorporació d'una coberta de lleguminosa. En tots els experiments s'ha avaluat simultàniament el funcionament de les associacions i els respectius monocultius.

La selecció de les varietats de blat i el cultiu de cobertura estudiats s'ha dut a terme mitjançant un procés participatiu amb els agricultors de l'Associació Agroecològica de Gallecs i els fariners locals, així com amb la direcció tècnica del Consorci de l'Espai d'Interès Natural (EIN) de Gallecs. L'EIN de Gallecs és una àrea rural periurbana de 698,91 ha situada a 15 km al nord de Barcelona (Mollet del Vallès) predominantment agrícola. Des del 2005, l'Associació Agroecològica de

Gallecs ha promogut activament un procés de reconversió i transició cap a l'agricultura ecològica, on destaca la sembra de varietats tradicionals de blats i lleguminoses (Chamorro et al. 2017).

La tria de les varietats de blat s'ha basat en criteris com la representativitat de l'ús de les varietats a Catalunya, el valor nutritiu, el valor cultural i el potencial de comercialització en el cas de la barreja de varietats de blat (Serra-Gironella 2020). Des d'una perspectiva científica, també s'ha tingut en compte els aspectes morfològics i fenològics i els atributs fisiològics, com ara la susceptibilitat als pugons, de les diferents varietats (Taula 1). Aquest enfocament basat en l'expertesa col·lectiva dels actors implicats assegura que les varietats seleccionades s'ajustin adequadament als objectius dels investigadors i al mateix temps propicia que els productors adoptin els policultius de blat dissenyats.

Les varietats escollides han estat tres varietats de blat d'hivern tou *Triticum aestivum* L. subsp. *aestivum* (Florence-Aurora, Xeixa i Montcada) i una varietat de blat d'hivern dur *Triticum turgidum* L. subsp. *durum* Desf. (Husn.) (Forment). Tot i que estrictament el blat Forment sigui una espècie diferent, per simplificar, d'ara endavant ens hi referirem com a varietat. Com a coberta de lleguminosa es va sembrar el melgó (*Medicago polymorpha* L.), triat per la seva adaptació al clima mediterrani i perquè el seu port prostrat el fan una excel·lent elecció com a cultiu de coberta.

Taula 1 Principals característiques morfològiques i fenològiques de les quatre varietats de blat sembrades en els experiments de la tesi doctoral.

Nom científic	Nom popular	Classificació varietal[1]	Característiques diferencials[2]	Alçada[3]	Fenologia[2]	Força farinera [3]	Qualitats en panificació [2]
Triticum aestivum L. subsp. *aestivum*	Florence-Aurora	Varietat moderna antiga (data dels anys 50)	Planta amb tija i fulles de tons glaucs. Espiga mútica	Port baix (aprox. 70 cm)	Data d'espigat primerenca	350 - 400 W	Farina de força excel·lent per a la panificació
Triticum aestivum L. subsp. *aestivum*	Xeixa	Varietat tradicional antiga	Planta glauca de lígules curtes. Espiga allargada amb arestes curtes. Sensible a l'ajagut	Port alt (aprox. 170 cm)	Data d'espigat tardana	43 W	Apreciat pel seu alt contingut en fibra
Triticum turgidum L. subsp. *durum* Desf. (Husn.)	Forment	Varietat tradicional antiga	Planta pubescent, de tacte suau amb lígules llargues. Espiga amb arestes llargues d'angle agut. Sensible a l'ajagut	Port alt (aprox. 180 cm)	Data d'espigat tardana	43 W	Alt valor aromàtic
Triticum aestivum L. subsp. *aestivum*	Montcada	Varietat tradicional vella (data dels anys 30)	Planta glauca amb lígules dentades. Espiga curta amb arestes llargues dipositades en angle obtús	Port baix (aprox. 70 cm)	Data d'espigat primerenca	100 W	Farina de força bona per a la panificació

1 Serra-Gironella (2020); 2 Web del Consorci de Gallecs; 3 Serra-Gironella & Àlvaro (2017).

Cada un dels quatre capítols que conformen el cos de la tesi doctoral adopta un enfocament únic per tractar aspectes de la recerca que han rebut menys atenció fins ara. Els dos primers capítols recullen els resultats de dos experiments en condicions naturals realitzats a l'EIN de Gallecs. El primer capítol s'ha establert en una parcel·la experimental permanent i el segon en cinc camps comercials, tots ells són camps on s'ha practicat l'agricultura ecològica des de l'any 2006, amb rotacions de cereals i lleguminoses.

El *capítol 1* respon a la necessitat d'aprofundir en el paper de les pràctiques emprades en el maneig del sòl sobre el funcionament del sistema agrícola (Tamburini et al. 2016). Per això, el treball estudia la incidència de la intensitat de llaurada (llaurada amb l'arada de pales, que causa inversió de les capes superficials del sòl, versus l'arada de cisell, sense inversió de les capes) i la fertilització (incorporació de fems compostats versus absència de fems) sobre l'abundància de les poblacions d'arvenses i de pugons, la taxa de parasitisme i l'estabilitat de la producció de gra en l'associació de tres varietats de blat (Florence-Aurora, Forment i Xeixa) i els respectius monocultius. L'estudi es va dur a terme en dos períodes de cultiu entre l'octubre de 2018 i el juny de 2022.

El *capítol 2* presenta els resultats d'un estudi en condicions naturals on es compara l'efecte de diferents policultius de blat sobre l'abundància de les poblacions de pugons i dels seus enemics naturals (predadors aeris i terrestres i parasitoides) i la producció de gra de cereal. L'experimentació també ha possibilitat l'avaluació del paper de la diversitat planificada sobre els mecanismes *bottom-up* i *top-down* involucrats en el control de plagues. Els policultius estudiats han estat dues associacions de dues varietats de blat, els respectius monocultius i la incorporació d'una coberta vegetal de melgó a cadascun d'aquests cultius. Una de les associacions estava formada per dues varietats de blat morfològicament i fenològicament similars, Florence-Aurora i Montcada, i l'altra per dues varietats amb trets funcionals diferents, Florence-Aurora i Forment. L'estudi es va dur a terme en dos períodes de cultiu entre l'octubre de 2019 i el juny de 2022.

Els dos darrers capítols incideixen en l'anàlisi dels mecanismes *bottom-up* de regulació de les poblacions de pugons observats en condicions naturals en l'estudi que es descriu en el *capítol 2.* Per aquest motiu, es caracteritzen els trets funcionals de les varietats de blat Florence-Aurora, Montcada i Forment i s'analitzen les interaccions (planta – planta i planta – pugó) en diferents fases del procés de selecció de l'hoste i desenvolupament de la colònia de pugons en condicions experimentals d'hivernacle i de laboratori. La part experimental d'aquests estudis s'ha dut a terme a les instal·lacions del grup de recerca d'Ecologia de Sistemes Agraris de la SLU (Universitat de Ciències Agrícoles de Suècia) entre el mes d'abril i juny del 2022.

El *capítol 3* mostra els resultats de l'efecte de la interacció planta-planta durant la fase d'acceptació de l'hoste i del desenvolupament de la colònia de pugons en experiments controlats de laboratori i hivernacle, respectivament. També s'ha caracteritzat i analitzat l'efecte de la biomassa aèria i el contingut de nitrogen tissular de les varietats de blat sobre l'abundància de pugons. Hi ha una àmplia literatura sobre la relació entre el nitrogen i les poblacions de pugons en els sistemes agrícoles. La major part de la recerca s'ha centrat en la relació entre la fertilització nitrogenada del sòl i l'abundància de pugons en monocultius de cereals (Duffield et al. 1997; Nowak & Komor 2010; Gash 2012). En canvi, hi ha pocs estudis que indaguin sobre les diferències en el contingut de nitrogen tissular de les varietats de cereals que formen part de les associacions, el paper que tenen en el control de les poblacions de pugons i si la interacció entre les varietats modifica la capacitat de controlar les poblacions de pugons.

El *capítol 4* correspon a un estudi exhaustiu sobre l'efecte dels senyals olfactius emesos per les plantes de blat en la fase de localització i selecció de l'hoste del procés d'infestació dels pugons. Primerament, s'ha comparat la composició i les proporcions dels volàtils que conformen el perfil olfactiu de les tres varietats crescudes en règim de monocultiu. Simultàniament, s'ha caracteritzat el perfil olfactiu de les barreges per analitzar la incidència de les interaccions planta-planta en aquest tret. Finalment, s'ha avaluat la interacció planta-pugó mitjançant l'anàlisi de la resposta d'atracció o repulsió dels pugons cap als senyals olfactius dels diferents monocultius i policultius. Per tal d'assegurar que la resposta dels pugons és causada únicament pels senyals olfactius, es van eliminar la resta d'estímuls sensorials que poden incidir en la localització i selecció de l'hoste, com ara els estímuls visuals, tàctils o tast de la qualitat de la saba (Glinwood et al. 2009).

Objectius

L'objectiu general de la tesi és estudiar diferents policultius de blat com a estratègia per a afavorir el control de les poblacions de pugons, la regulació de les poblacions d'espècies arvenses i la producció de gra. Els estudis en condicions naturals tenen la finalitat d'analitzar la influència de les pràctiques de maneig del sòl (intensitat de la llaurada i fertilització) en la funcionalitat de l'associació de tres varietats de blat vers els monocultius, i també d'avaluar la resistència associativa de barreges de varietats, amb trets morfològics i fenològics similars o diferents, i de la incorporació d'una coberta de lleguminosa als monocultius i barreges de blat. De manera complementària, s'han dut a terme estudis en condicions controlades d'hivernacle i laboratori per aprofundir en les interaccions planta-planta i planta-pugó observades en condicions naturals i avaluar el paper de trets funcionals essencials per a la resistència als pugons, com són la quantitat de nitrogen en el teixit vegetal, la biomassa aèria i el perfil olfactiu.

A continuació es detallen els objectius concrets dels quatre capítols que conformen la present tesi doctoral:

Capítol 1

- Avaluar l'efecte de l'associació de tres varietats de blat (Florence-Aurora, Forment i Xeixa) i els respectius monocultius sobre l'abundància de les poblacions d'espècies arvenses, de pugons, la taxa de parasitisme i la producció de gra en una parcel·la experimental permanent.

- Analitzar la incidència de la intensitat de la llaurada (l'arada de pales respecte de l'arada de cisell) i de la fertilització (la periòdica incorporació de fems compostats respecte a la no incorporació de fems) sobre l'abundància de les poblacions d'espècies arvenses, de pugons, la taxa de parasitisme i la producció de gra en una associació de tres varietats de blat, Florence-Aurora, Forment i Xeixa i en el monocultiu de cadascuna de les varietats en una parcel·la experimental permanent.

- Estudiar la influència de la intensitat de la llaurada i de la fertilització sobre l'efecte de l'associació de tres varietats de blat, Florence-Aurora, Forment i Xeixa i els respectius monocultius en l'abundància de les poblacions d'espècies arvenses, de pugons, la taxa de parasitisme i la producció de gra.

Capítol 2

- Avaluar l'efecte (i) d'una barreja de dues varietats de blat amb morfologia i fenologia similars, Florence-Aurora i Montcada (ii) d'una barreja de dues varietats amb morfologia i fenologia diferents, Florence-Aurora i Forment (iii) dels tres monocultius i (iv) de la incorporació d'una coberta vegetal de melgó als cinc cultius anteriors sobre l'abundància de pugons i la producció de gra.

- Estudiar el paper (i) d'una barreja de dues varietats de blat amb morfologia i fenologia similars, Florence-Aurora i Montcada, (ii) d'una barreja de dues varietats amb morfologia i fenologia diferents, Florence-Aurora i Forment (iii) dels tres monocultius i (iv) de la incorporació d'una coberta vegetal de melgó sobre els mecanismes *bottom-up* i *top-down* involucrats en el control de plagues mitjançant l'anàlisi del número de pugons per fillol, l'anàlisi de la riquesa i l'abundància artròpodes predadors i la taxa de parasitisme

Capítol 3

- Caracteritzar el contingut de nitrogen tissular i la biomassa aèria de tres varietats de blat, Florence-Aurora, Forment i Montcada, cultivades en monocultiu i associades (Florence-Aurora amb Forment i Florence-Aurora amb Montcada) en condicions controlades d'hivernacle.

- Analitzar la relació entre el desenvolupament de la població de pugons i el contingut de nitrogen tissular de tres varietats de blat, Florence-Aurora, Forment i Montcada cultivades en monocultiu o en associacions.

- Comparar l'efecte de dues associacions de varietats de blat (Florence-Aurora amb Forment i Florence-Aurora amb Montcada) sobre el control de la població de pugons durant la fase d'acceptació de l'hoste i el desenvolupament de la colònia de pugons.

Capítol 4

- Caracteritzar els perfils olfactius, composició i proporció de volàtils emesos, de tres varietats de blat, Florence-Aurora, Forment i Montcada, sembrades en monocultiu en condicions experimentals de laboratori.

- Analitzar l'efecte de la interacció entre les varietats de blat sobre el perfil olfactiu de la barreja que conformen, mitjançant la caracterització del perfil olfactiu de dues barreges (Florence-Aurora amb Forment i Florence-Aurora amb Montcada).

- Avaluar la resposta dels pugons, d'atracció o de rebuig, als senyals olfactius dels monocultius i les barreges de blat (Florence-Aurora amb Forment i Florence-Aurora amb Montcada) mitjançant el mètode de l'olfactòmetre.

Capítol 1

Wheat cultivar mixtures enhance the delivery of agroecosystem services compared to monocultures under contrasted tillage intensities and fertilization

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Wheat cultivar mixtures enhance the delivery of agroecosystem services compared to monocultures under contrasted tillage intensities and fertilization

Resum

L’associació de varietats de blat proporcionen un sistema més complex que els monocultius capaç d’oferir diversos serveis. En el present estudi hem avaluat la funcionalitat dels monocultius de la varietat moderna Florence-Aurora i de les varietats tradicionals Xeixa (*Triticum aestivum* L. subsp. *aestivum*) i Forment (*Triticum turgidum* L. subsp. *durum*) i de la seva barreja sobre el control de les poblacions d’espècies arvenses i de pugons, així com en la millora del rendiment. Els diversos cultius s’han sotmès a diferents intensitats de llaurada (llaurada amb arada de pala, que genera inversió del sòl, versus. llaurada de cisell) i fertilització (aplicació de fem compostat o sense aplicació). Els paràmetres analitzats van ser l'abundància de pugons, el nombre de pugons per fillol, la taxa de parasitisme, l'abundància i riquesa d’espècies arvenses i la producció del cultiu. A més, vam examinar l'establiment, la cobertura, la fenologia i l'alçada del blat per a la caracterització de les varietats.

Els resultats obtinguts indiquen que la llaurada de cisell incrementa l’abundància de les poblacions d’arvenses, mentre que la llaurada de pales augmenta la població de pugons. La varietat moderna Florence-Aurora va ser més susceptible als pugons i a la infestació. L’associació de varietats de blat va demostrar una disminució de la població de pugons en qualsevol intensitat de llaurada, en canvi, només es va observar una menor infestació d’herbes en condicions de llaurada de cisell.

La producció del monocultiu de Florence-Aurora i la barreja de varietats va estar influenciada per la llaurada i fertilització. Concretament, la producció va ser significativament inferior en les subparcel·les no fertilitzades i llaurades amb cisell. En conclusió, el nostre estudi revela que la intensitat de llaurada i la fertilització afecten les funcions proveïdes per l’associació de varietats de blat, i la importància d’investigar la funcionalitat dels policultius en condicions naturals per poder avaluar l’impacte de factors agronòmics, com ara la gestió del sòl.

1. Introduction

The establishment of monocultures and the substitution of traditional cultivars for modern ones remain popular for organic cereal crops of the Mediterranean basin (Di Bene et al. 2022; Serra-Gironella 2020). These modern cultivars, usually selected for their high yield (Ficiciyan et al. 2018), are more efficient than traditional cultivars under optimal environmental conditions, but they are more prone to pest infestation and are weaker competitors against weeds (Bonnet et al. 2021; Lazzaro et al. 2017; Serra-Gironella 2020). This agricultural framework has led to a simplification of the cropping system, including a significant loss of agrobiodiversity and associated diversity and an overall decline in agroecosystem functionality (Altieri & Rogé 2009; Dainese et al. 2019; Tilman et al. 2014).

In contrast, the increase in agrobiodiversity in cereal crops enhances agroecosystem complexity and functionality while maintaining natural biodiversity within the field (Altieri & Rogé 2009; Barot et al. 2017; Gaba et al.2015; Koricheva & Hayes 2018; Wezel et al. 2014). Following Serra-Gironella (2020), a cultivar mixture can be defined as the simultaneous sowing of a high-yield cultivar and one or a few companion cultivars, which offer specific benefits to the polyculture. Specifically, the implementation of cereal cultivar mixtures can provide resistance against pests and parasites, heightened competitiveness against weeds, and improved yield stability. These functions ultimately contribute to the provision of agroecosystem services (Costanzo & Bàrberi 2014; Gibson & Nguyen 2021; Tilman et al. 2014). In mixtures with a small number of cultivars, it is necessary to assess cultivar traits associated with target agroecosystem services to avoid functional redundancy and enhance mixture performance (Gaba et al. 2015; Lazzaro et al. 2018).

Aphids are considered a potential pest in cereal crops (Dedryver et al. 2010) and serve as a model organism for the study of insect-plant interactions (Rodriguez-Saona & Stelinski 2009). Functional mixtures of cereal cultivars offer aphid control services through associational resistance, wherein resistant cultivars visually and olfactorily mask the susceptible cultivar from aphids (Barbosa et al. 2009). For instance, Shoffner and Tooker (2013) conducted a laboratory experiment that found lower aphid abundance when mixing six-line wheat cultivars with varying levels of aphid resistance compared to three-line cultivar mixtures or monocultures. Moreover, cereal cultivar mixtures also provide aphid control through indirect means by potentially increasing the abundance of aphids' natural enemies, such as parasitoid wasps and predatory arthropods (Glinwood et al. 2009; Koricheva & Hayes 2018; Ninkovic et al. 2011). In regard to weed control services, cereal cultivars exhibit significant diversity in functional traits associated with weed suppression. For instance, wheat cultivars differ in characteristics such as height, aboveground biomass and tillering capacity. An increased diversity of wheat traits can build up a more competitive environment by capturing more light and a broader spectrum of nutrient resources, ultimately contributing to weed control services (Lazzaro et al. 2018; Worthington et al. 2013). Finally, the combination of traditional and modern wheat cultivars improves resilience to variation in environmental conditions, thereby enhancing yield stability (Borg et al. 2018; Ficiciyan et al. 2018; Reiss & Drinkwater 2018).

Nevertheless, most of the research on cultivar mixture functionality has been conducted within controlled settings, ignoring the complexity existing in real farming conditions (Barot et al. 2017; Gaba et al. 2015). In this regard, soil management practices, such as tillage and soil fertilization, can alter the cropping system performance by modifying weed abundance and nitrogen availability, which in turn may influence the functionality of cultivar mixtures (Tamburini et al. 2016). On the one hand, conventional tillage (e.g. moldboard ploughing) is a common practice for reducing weed infestations (Armengot et al. 2015; Sans et al. 2011). Weeds can affect crop growth and yield (Baldivieso-Freitas et al. 2018; Diacono et al. 2019; Tamburini et al. 2020). However, it can negatively affect weed species richness (Tamburini et al. 2016). On the other hand, nitrogen fertilization is a crucial factor for crop yield in cereal crops (Baldivieso-Freitas et al. 2018; Tamburini et al. 2016). However, nitrogen-rich soil conditions can boost aphid infestation because of the crop's high-quality sap (Aqueel & Leather 2011; Chabert & Sarthou 2017; Nowak & Komor 2010) (Figure 1).

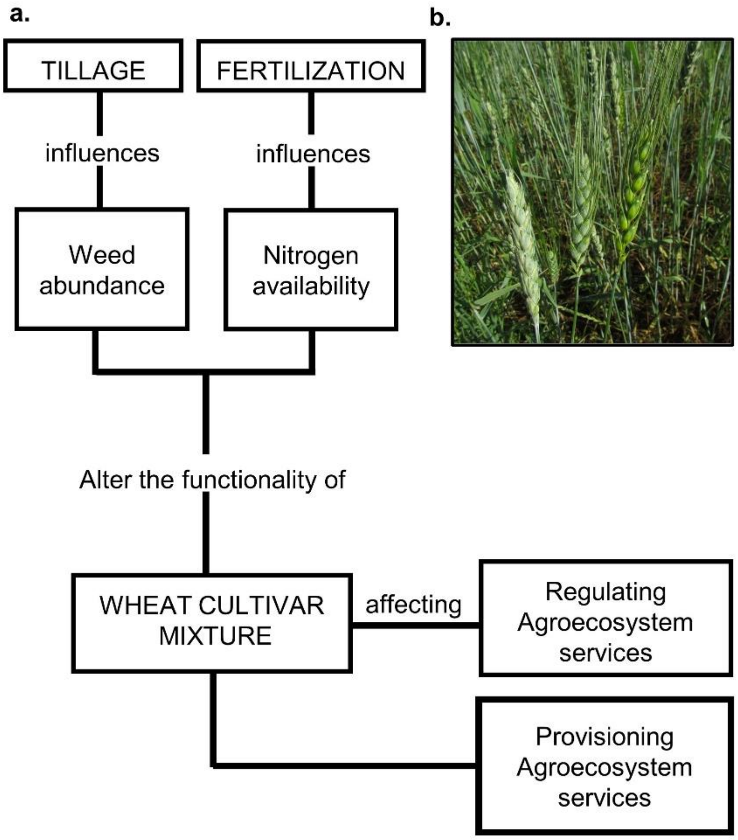

Figure1 a. Effect of tillage practices and fertilization on the ability of cultivar mixtures to provide services to the crop system. b. Three wheat cultivars used in the mixture treatment. Left to right: Florence-Aurora, Forment and Xeixa.

The aim of this study was to assess the influence of soil management practices, specifically contrasting tillage systems and fertilization, on the functionality of different wheat varieties, particularly their mixture, in a long-term organic arable field experiment. We evaluate the performance of three wheat cultivar monocultures (one modern and two traditional) and the resulting cultivar mixture in providing aphid and weed control, as well as crop yield stability under different tillage and fertilization practices. We hypothesize that (i) the three cultivars exhibit distinct functional traits associated with weed and aphid control, (ii) the cultivar mixture displays lower weed infestation compared to monocultures, (iii) the cultivar mixture offers aphid control by both associational resistance and increased parasitism rate, (iv) the cultivar mixture and even traditional cultivar monocultures present yield stability, and (v) tillage practices and fertilization affect the functionality of the cultivar mixture.

2. Materials and methods

2.1. Study site

This study was performed during two cropping seasons (2018-2019 and 2020-2021) in the rural area of Gallecs, which is a periurban agricultural area of 755 ha located 15 km north of Barcelona (41°33'31.9" N 2°11'59.5" E, Catalonia, Northeast Spain). The area has a Mediterranean climate with hot and dry summers and mild winters, with a mean annual temperature of 14.6 °C and 629.2 mm mean annual rainfall. During the two cropping seasons, from December to June, the mean temperature and accumulated precipitation were 13.7 °C and 132.5 mm in 2018-2019 and 13.2 °C and 83.8 mm in 2020-2021 periods, respectively (Table 1).

The study was carried out in the long-term Gallecs trial, an experiment established in 2011 combining contrasted tillage and fertilization factors over a cereal–legume arable crop rotation. The crops preceding the first and second sampling seasons were the vetch-oat mixture crop and chickpea crop, respectively. Since 2006, the field has been managed organically, with no application of pesticides or herbicides.

In 2011, we analyzed the soil properties of the field. The soil was a loamy clay (Baldivieso-Freitas et al. 2018) with low soil organic matter (1.5 ± 0.1%) and a slightly alkaline nature (pH = 8.1 ± 0.1). In 2020, the total nitrogen soil content was assessed. The average values were 0.179 ± 0.006 % in fertilized plots and 0.133 ± 0.006 % in non-fertilized ones.

Table 1 Monthly mean temperature and precipitation from the nearest automatic weather station (Parets del Vallès, 41° 34’ 2.4” N 2° 13’ 34.2” E) for the wheat growing season in 2018–2019 and 2020–2021.

	2018- 2019						
	DEC	JAN	FEB	MAR	APR	MAY	JUN
Temperature (ºC)	16.1	6.3	9.4	12.0	13.5	16.1	22.6
Precipitation (mm)	4.8	12.9	0.8	1.9	30.0	68.8	13.3
	2020-2021						
	DEC	JAN	FEB	MAR	APR	MAY	JUN
Temperature (ºC)	8.8	7.1	11.5	11.4	12.9	17.6	23.4
Precipitation (mm)	80.1	0.5	0.8	0.4	1.0	0.8	0.2

2.2. Selection of winter wheat cultivars

The three wheat cultivars used in the experiment were selected in collaboration with local farmers. We selected a modern cultivar, Florence-Aurora (*Triticum aestivum* L. subsp. *aestivum*), which is the predominant cultivar sown in the organic fields of Gallecs, and two traditional cultivars, Xeixa (*Triticum aestivum* L. subsp. *aestivum*) and Forment (*Triticum turgidum* L. subsp. *durum* Desf. (Husn.)). The modern cultivar, Florence-Aurora, is widely known for its baking qualities, while the other two impart aroma to the bread. The flour of the three cultivars is already mixed for bread manufacturing in mills.

Furthermore, the traditional cultivars have different functional traits compared to Florence-Aurora. The three cultivars adopt an erect growth habit early in their vegetative stages. However, Xeixa and Forment can present lodging problems at the ripening stage (Serra-Gironella & Álvaro 2017). In addition, Florence-Aurora is semidwarf tall (70 cm at harvest time), whereas Forment and Xeixa are traditional tall (180 cm at harvest time) (Serra-Gironella & Álvaro 2017). Plant height is a trait related to weed control (Lavorel and Garnier 2002). Concerning aphid susceptibility, laboratory studies on odor profiles have indicated that Florence-Aurora was more attractive to aphids than traditional winter wheat cultivars (Tous-Fandos et al. 2023). Moreover, flour strength is 350 W, 43 W, and 43 W in Florence-Aurora, Xeixa, and Forment, respectively (Serra-Gironella & Álvaro 2017). Aqueel & Leather (2011) demonstrated in a laboratory experiment that some aphid species (e.g., *Sitobion avenae* (Fabricius, 1775) and *Rhopalosiphum padi* (Linnaeus, 1758)) were healthier and more fertile when feeding on high flour strength cultivars and thus could reach higher population densities on these cultivars. Finally, the average yield of the three monocultures in the area at a sowing rate of 200 kg/ha are 1800 kg/ha for Florence-Aurora and 1400 kg/ha for Xeixa and Forment.

2.3. Experimental design

The experimental design was a strip-split block with three factors: tillage (conventional tillage using moldboard (MB) versus reduced tillage employing chisel (C)), fertilization (application of composted farmyard manure (F) versus no fertilizer (NF)), and crop type (three monocultures: Florence-Aurora (FA), Xeixa (XE) and Forment (FO), and the mixture of the three cultivars (MIX)).

The tillage factor was laid out in eight vertical strips, alternating conventional and reduced tillage (four tillage blocks). Each vertical strip consisted of two contiguous plots. The fertilization factor was applied in two horizontal strips randomly across each two vertical strips (four fertilization blocks), totaling 16 plots measuring 12 m × 26 m. The 16 plots were divided into four subplots, creating 64 subplots of 6 m × 13 m. Each subplot was sown with one of the four crop type treatments considering the strip-split block design and the agricultural machinery operability. Ultimately, we had four replicates for each combination of tillage, fertilization, and crop treatment (Figure SM 1).

We used a moldboard plough (EG 85-240-8, Kverneland) for the conventional tillage treatment, which turned the soil over at a depth of 25 cm. In contrast, we used a chisel plough (KCCC 1187 – A00, Kverneland) for the reduced tillage treatment, operated at the same depth but with no soil inversion. In both cases, a rotary harrow at 5 cm depth was used to prepare the seedbed. Fertilized plots received 140 kg N ha-1 of manure. Tillage and manure application were performed annually between November to January, prior to sowing. No fertilizer plots received no fertilization since 2011.

The sowing rates of FA, XE, FO, and MIX were 200 kg ha-1 in both years. The sowing ratio for the MIX was 6FA:2XE:2FO. Due to the greater yield capacity and higher thousand kernel weight (TKW) of the Florence-Aurora cultivar, we sowed 110 kg/ha of FA and 55 kg/ha each of XE and FO in the mixture to achieve the desired ratio. The ratio of cultivars was equivalent to the ratio of cultivar flour when combined for bread manufacturing. Organic seeds treated with copper to prevent fungal infestation were provided by local farmers.

2.4. Field sampling

2.4.1. Wheat cultivar growth and phenology

At the stem elongation stage at mid-February both years, we analyzed wheat establishment by counting the number of wheat plants on both sides of two 50 cm linear samples aligned to sowing lines, randomly placed in every subplot, 2 m from the subplot edge. Two weeks before harvest time, we assessed wheat cover. The wheat cover was estimated visually by well-trained samplers in one 1 m2 quadrat randomly placed at least 2 m away from the edge in each subplot following previous evaluations (Baldivieso-Freitas et al. 2018).

In 2019, wheat height and phenology samplings were carried out weekly from early April to mid-June. We collected and measured the plant height and phenology of ten wheat tillers evenly distributed along a 10 m transect situated in the middle of the subplot. The wheat height was measured from the base to the top of the stem. From the booting stage, height was measured from the base to the flag leaf. Wheat phenology was assessed visually using Zadoks' scale (Zadoks et al. 1974). In the cultivar mixture, wheat cultivars were distinguished. Therefore, the number of tillers collected for each cultivar corresponded to the initial sowing ratio. We calculated the mean and standard error for height and phenology stage per wheat cultivar and date.

Wheat height was analyzed as an important trait for weed suppression (Worthington et al. 2013). On the other hand, phenology was evaluated to determine the feasibility of harvesting the three cultivars simultaneously when mixed in real farming conditions. In addition, fast developing cultivars have shown good weed suppression abilities (Coleman et al. 2001).

2.4.2. Weed species richness and abundance

Weed abundance was estimated by the aboveground dry biomass of weeds at harvest time which was Late-June at both sampling seasons. The aboveground biomass was hand-harvested in two 0.25 m² quadrats randomly distributed per subplot, 2 m away from the edge to avoid edge effects. The collected samples of aboveground biomass were oven-dried for 48 h at 60 °C. Two weeks before harvest time weeds were identified and counted for weed species richness assessment in one 1 m2 quadrat at least 2 m away from the edge (Baldivieso-Freitas et al. 2018).

2.4.3. Aphid abundance and parasitism rate

The sampling of cereal aphids and parasitoids was carried out every three weeks from early April to mid-June during the two cropping seasons. We counted aphids and mummies (parasitized aphids) on 10 wheat tillers evenly distributed along a 10 m transect in the center of the subplots (Mansion-Vaquié et al. 2017). In the cultivar mixture subplots, we differentiated between wheat cultivars according to the intended sowing ratio (6 tillers of FA and 2 each of XE and FO), and aphids were counted separately by cultivar. The aphid species were identified in the field. In 2021, unhatched mummies were reared in vials until parasitoids emerged. Both the parasitoid adults and the aphid mummies were then preserved in 70% ethanol. We identified hatched parasitoids at the genus level.

In this experiment, we analyzed aphid abundance, number of aphids per tiller, and parasitism rate. Aphid abundance was determined by the total sum of aphids recorded per subplot during the sampling season. It expressed the overall aphid pressure in every treatment. The number of aphids per tiller was examined at the aphid population peak, which was early May both years. It was defined as the number of aphids on a cultivar tiller grown in monoculture (FA, XE or FO) or in the cultivar mixture (MIX). Hence, it allowed the comparison of aphid infestation on the same cultivar when grown in different crop types. The number of aphids per tiller was used as an indicator of the effects of associational resistance on the preferred cultivar. Finally, the parasitism rate was calculated by dividing the cumulative number of mummies by the total number of non-mummified and mummified aphids per subplot. The parasitism rate was assessed and analyzed to test for cascading effects.

2.4.4. Crop yield

The total dry grain weight per subplot was obtained by a plot combine harvester and weighed on-site with a crane scale at the end of June. In addition, grain aliquots (800 to 1000 g) were cleaned in the laboratory, and oven-dried (48 h at 60 °C) to extrapolate grain dry weight.

The land equivalent ratio (LER) was used to determine the yield advantage of the cultivar mixture over sole crops. LER can be defined as the total land area of sole crops needed to achieve the same yield as polycultures (Willey and Rao 1980):

$$\mathrm{LER}=\frac{Y_{\mathrm{MIX}}}{Y_{\mathrm{FA}}\times Z_{\mathrm{FA}}+Y_{\mathrm{XE}}\times Z_{\mathrm{XE}}+Y_{\mathrm{FO}}\times Z_{\mathrm{FO}}}$$

where YMIX, YFA, YXE and YFO are the yields of wheat in MIX and the sole crop, respectively; ZFA, ZXE and ZFO are the sowing ratios in the cultivar mixture. An LER larger than 1.0 means that cultivar mixtures benefit crop yield, whereas values less than 1.0 imply that cultivar mixtures negatively affect the yield of wheat.

2.5 Statistical analysis

All statistical analyses were conducted using R, version 4.1.1 (R Development Core Team 2021). The variables wheat establishment, wheat cover, wheat height, weed abundance, weed richness, aphid abundance, number of aphids per tiller, total parasitism rate, and crop yield were analyzed with linear mixed effects models (LMM) or generalized linear mixed effects models (GLMM) using the *glmmTMB* package (Brooks et al. 2017).

For each response variable, we fitted fifteen models encompassing all potential interactions. These models included four fixed factors: tillage, fertilization, crop type, and year (2019 and 2021). Additionally, tillage and fertilization blocks were included as random factors to account for the blocking structure. Wheat cover was incorporated as a covariate in weed biomass, aphid abundance, and yield models. After exploring all possible combinations, the best model for every response variable was selected according to its Akaike information criterion corrected for small sample sizes (see Table 2). We addressed spatial dependence in the residuals by incorporating principal coordinates of neighbor matrices (PCNM) (Borcard et al. 1992) when needed.

The significance of fixed effect factors and their interactions was determined with an F test with Kenward-Roger approximation for degrees of freedom for LMMs or a likelihood ratio test (LRT) for GLMMs. Pairwise comparisons were carried out using Tukey-adjusted estimated marginal means from the *emmeans* package (Lenth 2019). Post-hoc tests were performed independently for each year. The simulateResiduals function from the *DHARMa* package was utilized to examine overdispersion and residual distribution (Hartig 2019).

Wheat establishment, cover and height were fit in a Gaussian LMM without interactions. For statistical analysis we only consider wheat height at harvest time. Weed richness was fitted in a Gaussian LMM model with crop × year interaction. Weed biomass was analyzed using a gamma GLMM with a logit link function. The best fitted model had a crop × tillage interaction. Aphid abundance was fitted in a negative binomial model with year × crop type interaction. For the statistical analysis of number of aphids per tiller, each cultivar was analyzed independently. Hence, we categorized the crop factor into two distinct levels: monoculture (FA, XE, or FO) and MIX. To equalize the sowing ratios between monocultures and mixtures, the mean number of aphids on Florence-Aurora, Xeixa, and Forment tiller grown in the MIX were adjusted by dividing them by 0.6, 0.2, and 0.2, respectively. Then, the number of aphids per tiller was fitted to a negative binomial GLMM model without interactions. The parasitism rate was analyzed using a binomial GLMM with a logit link function. The best fitted model had a year × crop × fertilization triple interaction. Crop yield was assessed using a Gaussian LMM with a logarithmic link function. The best fitted model presented a crop type × year × tillage interaction.

3. Results and discussion

3.1. Winter wheat cultivar growth pattern and phenology

In this study, we combined the main modern cultivar, Florence-Aurora, with two traditional cultivars that demonstrated complementary traits for aphid control service, including cultivar odor profile and flour strength (Serra-Gironella & Álvaro 2017; Tous-Fandos et al. 2023). Furthermore, we assessed plant establishment, height, and phenology as key traits for weed infestation control and yield services (Costanzo & Bàrberi 2014; Lazzaro et al. 2018).

The mean wheat establishment was higher in 2019 than in 2021 ($F_{1,11}$ = 224.87, P < 0.001). The mean wheat establishment values (mean ± SE) were 59.5 ± 3.1 and 34.6 ± 6.0 individuals/m, respectively. Wheat establishment was different depending on crop type ($F_{3,9}$ = 51.12, P < 0.01). In 2019, wheat establishment was similar across crop treatments. However, in 2021, the FA wheat establishment was 21.4% lower than that of the other crop treatments.

Although the wheat establishment was similar between cultivars, crops presented different wheat cover before harvest time. Wheat cover values (mean % ± SE) were 39.5 ± 3.1 in FA, 47.9 ± 3.6 in XE, 49.2 ± 3.5 in MIX and 56.5 ± 3.8 in FO, being Florence-Aurora cover significantly lower than Forment in both years ($F_{3,61}$ = 228.99, P < 0.001). Hence, wheat canopy development differed across crops. These differences in wheat cover may lead to variations in aphid and weed pressure, affecting the ability of crops to respond to these stressors. Furthermore, wheat cover was significantly lower in every treatment in 2021 (F1,63 = 2778.12, P < 0.001). Finally, conventional tillage practices and fertilized treatments exhibited significantly higher wheat cover (F1,63 = 36.60, P < 0.05 and F1,63 = 26.60, P < 0.05, respectively).

Wheat cultivars differed in plant height ($F_{1,63} = 4.14$, $P < 0.05$). Specifically, the Florence-Aurora height (53.4 ± 2.7 cm) was significantly shorter than the Forment height (81.4 ± 4.5 cm) at harvest time. Xeixa presented an intermediate height (62.5 ± 3.1 cm) (Figure 2). The height of the cultivars showed no variation between individuals cultivated in monoculture and those cultivated in a mixture. This pattern suggests little intraspecific competition for light and nutrient resources, which is favorable for attaining high yields (Lavorel and Garnier 2002). Interestingly, neither tillage nor fertilization influenced plant height. Our data may hint that soil management practices might not have a direct effect on mixture performance, as cultivar target traits such as plant height and establishment remained constant.

The phenological analysis indicated that Florence-Aurora is the earliest cultivar to reach both flowering and grain ripening, followed by Xeixa and then Forment. The lag between Florence-Aurora and the other two cultivars was approximately three to four weeks for both flowering and ripening, while between Xeixa and Forment, it was just one week. All three cultivars reached full ripeness within a week from each other (Figure SM 2). This synchronized ripening permits the simultaneous harvest of all three cultivars without complicating agricultural management (Di Bene et al. 2022).

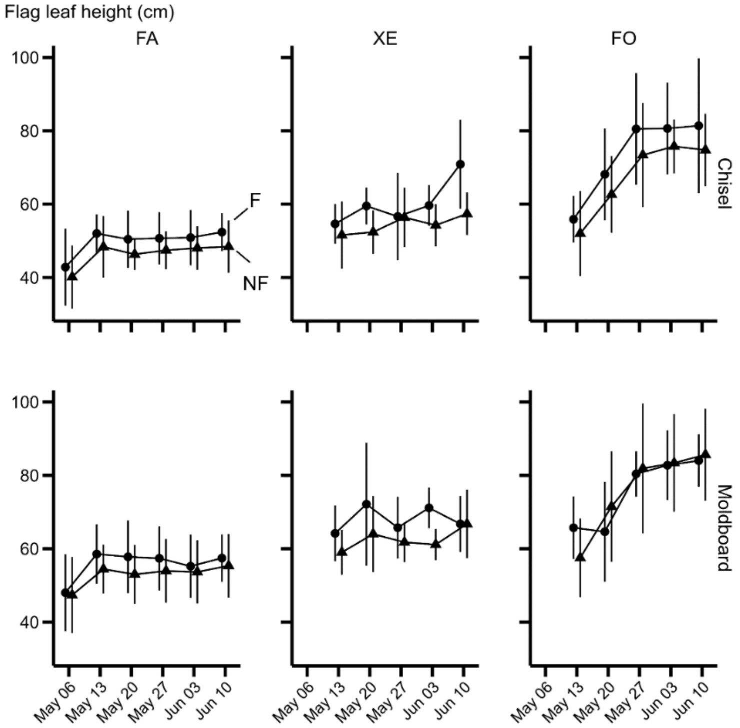

Figure 2 Wheat cultivar flag leaf height (mean ± SE) from May to June 2019 compared between fertilization (fertilizer (F) and no fertilizer (NF)) and tillage (chisel, moldboard). Wheat cultivars: Florence-Aurora (FA), Xeixa (XE), and Forment (FO).

Table 2 Results from generalized linear mixed models (GLMM) relating four factors: crop type (W), year (Y), Tillage (T) and Fertilization (F) and wheat cover as a covariable. The table includes only the interactions found in the best models. The significance of fixed effect factors and their interactions was determined with an F test with Kenward-Roger approximation for LMMs or a likelihood ratio test (LRT). Tillage, cultivar, and fertilization blocks were introduced as nested random factors. Wheat cover was added as covariable in weed biomass, aphid abundance and crop yield models. Only the best model for each response variable is presented.

Response variable	**Crop type (W)**	**Year (Y)**	**Tillage (T)**	**Fertilization (F)**	**Wheat cover**	**W×Y**	**W×T**	**W×T×Y**
Wheat establishment	$F_{3,9} = 51.12$ $P < 0.01$	$F_{1,11} = 224.87$ $P < 0.001$	$F_{1,11} = 2.17$ $P = 0.14$	$F_{1,11} = 0.22$ $P = 0.63$	-	-	-	-
Wheat cover	$F_{3,61} = 228.99$ $P < 0.001$	$F_{1,63} = 2778.12$ $P < 0.001$	$F_{1,63} = 36.60$ $P < 0.05$	$F_{1,63} = 26.60$ $P < 0.05$	-	-	-	-
Wheat height	$F_{1,63} = 4.14$ $P < 0.05$	-	$F_{1,63} = 0.11$ $P = 0.72$	$F_{1,63} = 3.18$ $P = 0.07$	-	-	-	-
Weed biomass	$F_{3,9} = 17.26$ $P < 0.001$	$F_{1,11} = 15.52$ $P < 0.001$	$F_{1,11} = 42.30$ $P < 0.001$	$F_{1,11} = 3.53$ $P = 0.06$	$F_{1,11} = 6.23$ $P < 0.05$	-	$F_{3,9} = 8.46$ $P < 0.05$	-
Weed richness	$F_{3,9} = 5.12$ $P = 0.09$	$F_{1,11} = 129.36$ $P < 0.001$	$F_{1,11} = 19.20$ $P < 0.001$	$F_{1,11} = 3.15$ $P = 0.14$	-	$F_{3,9} = 4.08$ $P = 0.11$		-
Aphid abundance	$\chi_{3,10} = 238.81$ $P < 0.001$	$\chi_{1,12} = 13.75$ $P < 0.001$	$\chi_{1,12} = 6.82$ $P < 0.05$	$\chi_{1,12} = 2.41$ $P = 0.08$	$\chi_{1,12} = 2.69$ $P = 0.10$	$\chi_{3,10} = 60.46$ $P < 0.001$	-	-
Parasitism rate	$F_{3,16} = 60.75$ $P < 0.001$	$F_{1,18} = 368.58$ $P < 0.001$	$F_{1,18} = 0.18$ $P = 0.67$	$F_{1,18} = 5.60$ $P = 0.14$	-	$F_{3,16} = 310.04$ $P < 0.001$	-	$F_{1,18} = 7.20$ $P = 0.08$

Num, aphids per FA tiller	$\chi_{1,5}$= 68.50	$\chi_{1,5}$= 11.10	$\chi_{1,5}$= 5.94	$\chi_{1,5}$= 0.06	-	-	-	-
	$P < 0.001$	$P < 0.001$	$P = 0.07$	$P = 0.80$				
Num, aphids per XE tiller	$\chi_{1,5}$ = 0.07	$\chi_{1,5}$ = 2.20	$\chi_{1,5}$= 0.55	$\chi_{1,5}$= 0.11	-	-	-	-
	$P = 0.78$	$P = 0.15$	$P = 0.41$	$P = 0.60$				
Num, aphids per FO tiller	$\chi_{1,5}$ = 1.25	$\chi_{1,5}$ = 5.65	$\chi_{1,5}$ = 1.09	$\chi_{1,5}$ = 0.15	-	-	-	-
	$P = 0.26$	$P = 0.07$	$P = 0.33$	$P = 0.70$				
Crop yield	$F_{3,61} = 7.80$	$F_{1,63} = 11.24$	$F_{1,63} = 9.31$	$F_{1,63} = 23.61$	$F_{1,63} = 17.84$	$F_{3,61} = 15.73$	$F_{1,63} = 4.68$	$F_{1,63} = 4.71$
	$P = 0.13$	$P < 0.01$	$P < 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.01$	$P = 0.19$	$P = 0.17$

3.2. Weed control service

In organic fields, weed control is a major concern for farmers. Reduced tillage practices enable biodiversity preservation but can result in weed infestations. On the other hand, conventional tillage practices control weed infestations but represent a threat to the diversity reservoir (Armengot et al. 2015; Baldivieso-Freitas et al. 2018). Moreover, we evaluated the potential of the wheat cultivar mixture for weed control as an alternative to weed suppression while conserving weed species richness.

The most common weed species identified over the course of the two sampling seasons were as follows: *Chenopodium album* at 63.3%, *Fallopia convolvulus* at 17.2%, *Lolium rigidum* at 5.3%, *Polygonum aviculare* at 2.4%, and *Kickxia spuria* at 1.9% of the total plant abundance recorded. Weed species richness was significantly affected by year ($F_{1,11}$ = 129.36, $P < 0.001$) and tillage ($F_{1,11}$ = 19.20, $P < 0.001$) factors. Conventional tillage significantly reduced weed species richness in both years (4.8 ± 0.3 and 9.0 ± 0.3 species per sample in 2019 and 2021, respectively) in comparison to reduced tillage (7.0 ± 0.4 and 9.9 ± 0.4), emphasizing the importance of reduced tillage practices to preserve weed diversity.

The weed abundance responded significantly to year ($F_{1,11}$= 15.52, $P < 0.001$), tillage ($F_{1,11}$ = 42.30, $P < 0.001$), crop × tillage interaction ($F_{3,9}$ = 8.46, $P < 0.05$) and wheat cover ($F_{1,11}$ = 6.23, $P < 0.05$). In contrast, fertilization had no influence on weed abundance ($F_{1,11}$ = 3.53, $P = 0.06$). Weed abundance doubled in 2021. On the other hand, conventional tillage led to a 22.14% reduction in weed biomass in 2019 and a 19.89% reduction in 2021 compared to plots with reduced tillage. Weed abundance was similar across crop types in 2019 but differed in 2021. Specifically, FA had a significantly higher weed abundance under reduced tillage than XE, FO and MIX (Figure 3).

Soil management practices had a significant effect on weed infestation, with tillage being the most influential factor. Previous studies have demonstrated that conventional tillage is efficient for weed control (Armengot et al. 2015; Baldivieso-Freitas et al. 2018; Sans et al. 2011). Furthermore, our findings indicate that soil nitrogen availability has a limited effect on weed control.

The provision of weed control by traditional cultivar monocultures or cultivar mixtures depended on tillage conditions. Crop factors had a significant impact under the higher weed infestation conditions generated by reduced tillage, but there were no differences between crop types under low weed infestation conditions as created by conventional tillage. Traditional taller cultivars presented greater wheat cover and were better at suppressing weeds than the modern and shorter Florence-Aurora cultivar, highlighting the importance of height as a plant trait for crop cover and weed suppression (Lazzaro et al. 2018; Worthington et al. 2013). Moreover, this study represents the first time that a three-cultivar wheat mixture effectively controls weeds in field conditions. Cultivar mixture was more competitive against weeds than the modern cultivar monoculture probably due to complementary heights and a greater wheat cover (Costanzo &

Bàrberi 2014; Lazzaro et al. 2018). Contrary to Coleman et al. (2001) investigations, Florence-Aurora early flowering did not provide benefits on weed suppression.

Our results were not consistent across sampling seasons. The wheat mixture effectively reduced weed infestation compared to the FA only during the 2020-2021 sampling season. In 2021, there were exceptionally dry growing conditions, as the region experienced approximately 80% reduction in rainfall. These conditions could explain the poorer crop establishment and cover particularly of the modern cultivar monoculture, consequently resulting in greater weed infestation in FA plots. In this context, the mixture of modern cultivars with traditional enables a stability of wheat establishment and posterior wheat cover that prevented high weed infestation.

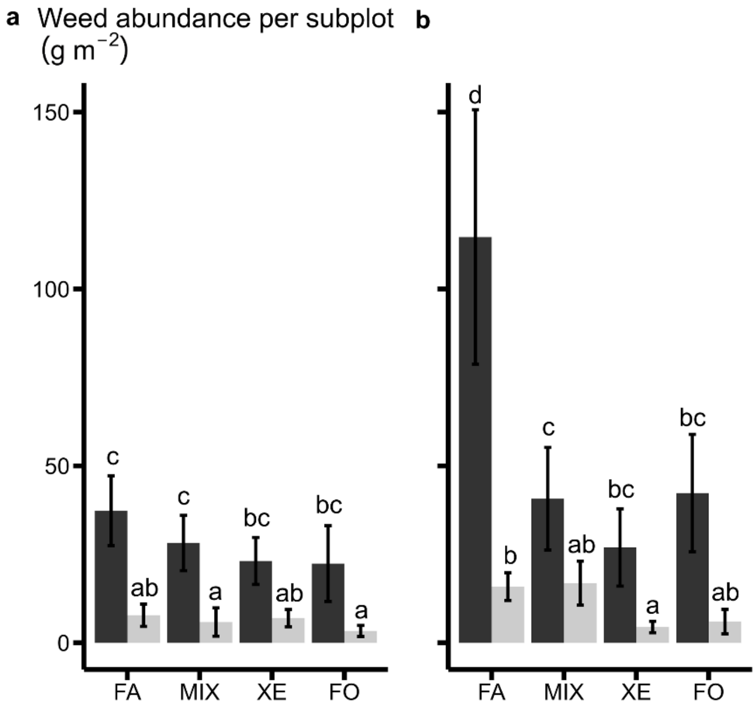

Figure 3 Aboveground dry weed biomass per subplot (mean ± SE) at harvest time in 2019 (a) and 2021 (b) according to crop type (Florence-Aurora (FA), Xeixa (XE), and Forment (FO) monocultures and mixture (MIX)) and tillage (chisel (black bars), moldboard (grey bars)) factors. Different letters above bars indicate statistically significant differences according to Tukey-adjusted pairwise EMMS comparisons ($P<0.05$) within each year.

3.3. Aphid control service

In this study, we also evaluated the effect of soil management practices on aphid abundance and the possible influence on cultivar mixture capacity for aphid control. During the two sampling seasons, we recorded a total of 39,275 aphid individuals on 3,840 wheat tillers. The main aphid species were *Sitobion avenae* 83.67%, *Metopolophium dirhodum* 11.27% and *Rhopalosiphum padi* 1.91%. Moreover, in the laboratory, we identified six parasitoid wasp genera, with *Aphidius* (48.34%) dominating as parasitoids and *Pachyneuron* (25.98%) and *Asaphes* (17.52%) genera as hyperparasitoids. The peak in aphid populations occurred in early May during both seasons, which coincided with the ear emergence phenology stage. This could be explained because the predominant aphid species, *Sitobion avenae*, generally colonizes cereal ears.

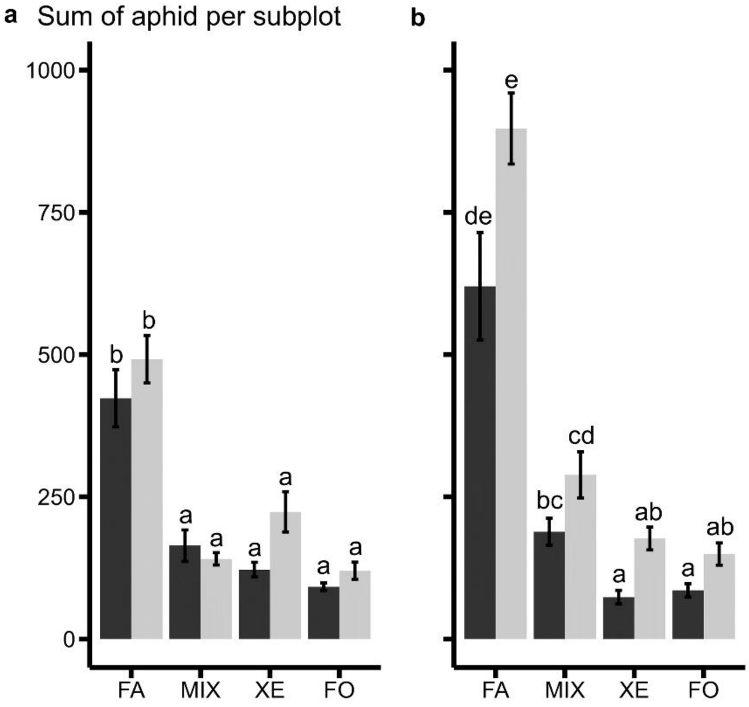

Figure 4 Overall sum of aphids per subplot (mean ± SE) in 2019 (a) and 2021 (b) according to crop type (Florence-Aurora (FA), Xeixa (XE), and Forment (FO) monocultures and mixture (MIX)) and tillage (chisel (black bars), moldboard (grey bars)) factors. Different letters above bars indicate significant differences according to Tukey-adjusted pairwise EMMS comparisons (P<0.05) within each year.

Aphid abundance showed significant differences according to year ($\chi_{1,12} = 13.75$, $P < 0.001$), crop ($\chi_{3,10} = 238.81$, $P < 0.001$), tillage factors ($\chi_{1,12} = 6.82$, $P < 0.05$), and crop × year interaction ($\chi_{3,10} = 60.46$, $P < 0.001$). Contrary to our expectations, neither fertilization ($\chi_{1,12} = 2.41$, $P = 0.08$) nor wheat cover ($\chi_{1,12} = 2.69$, $P = 0.10$) influenced aphid abundance. Aphid abundance was higher in 2021. Annual variation could be attributed to environmental stressors such as spring drought (see Table 1), which has also been found in similar field experiments (Mansion-Vaquié et al. 2019). However, more importantly, FA exhibited a consistently greater aphid abundance when compared to XE, FO or MIX in both sampling seasons. Furthermore, aphid abundance tended to increase with conventional tillage, although this trend was only significant in 2021 (Figure 4).

The number of aphids per tiller did not present a significant effect of tillage or fertilization factors. The number of aphids per tiller on Florence-Aurora tillers was significantly reduced in individuals grown in MIX ($\chi_{1,5}= 68.50$, $P<0.001$), whereas the number of aphids per tiller of Xeixa and Forment tillers was not influenced by crop type ($\chi_{1,5} = 0.07$, $P = 0.78$, and $\chi_{1,5} = 1.25$, $P = 0.26$, respectively) (Figure 5).

The parasitism rate displayed significant differences between the two years ($F_{1,18} = 368.58$, $P < 0.001$), with a rate of 16.0 ± 1.2% in 2019 and 12.5 ± 0.7% in 2021. Moreover, crop factor ($F_{3,16} = 60.75$, $P < 0.001$) and crop × year interaction ($F_{3,16} = 310.04$, $P < 0.001$) significantly affected the parasitism rate. However, it did not show any pattern. For instance, in 2019, the FA parasitism rate was 22.4 ± 1.3% and MIX 19.2 ± 0.9% ($P < 0.001$), while in 2021, the rates were 9.0 ± 0.5% in FA and 11.3 ± 0.7% in MIX ($P <0.01$).

Our research indicates that soil management practices altered aphid population abundance. In particular, higher aphid infestation in the conventional tillage treatment may be associated with the loss of weed cover and richness. This may lead to a decrease in within-field habitat quality for beneficial arthropods, such as aphid ground- and foliage-dwelling predators (Chabert and Sarthou 2020; Tamburini et al. 2016). Nonetheless, soil management practices did not influence the functions of traditional cultivar monocultures or cultivar mixtures concerning aphid control, as the cultivar mixture exhibited lower aphid populations compared to FA, irrespective of the tillage and fertilization factors.

The primary factor affecting aphid abundance was crop type (Costanzo & Bàrberi 2014; Tooker & Frank 2012). There was a notable difference in aphid susceptibility among cultivars, with the modern cultivar Florence-Aurora being more susceptible than the traditional cultivars Xeixa and Forment. Regarding cultivar mixtures, our research supported that combining modern aphid-susceptible cultivars with aphid-resistant traditional cultivars offered aphid control. The number of aphids per tiller results hinted that this control was likely achieved through associational resistance, as the Florence-Aurora plants grown in the mixture showed significantly lower infestations than the plants grown in monoculture. Associational resistance may have arisen because the selected cultivars had complementary functional traits, including distinct odor profiles, that can attract or repel aphids (Tous-Fandos et al. 2023), and differences in height and plant nitrogen content (Ninkovic et al. 2016; Nowak & Komor 2010). Moreover, aphid-resistant cultivars, Forment and Xeixa, were taller than the susceptible cultivar, which may impede aphid host localization (Barbosa et al. 2009).

Finally, the inconsistency of the parasitism rate and the large variation between years suggest that crop type, fertilization, or tillage were not relevant to the parasitism rate. Moreover, contrary to our hypothesis, it is improbable that the reduced aphid abundance observed in the cultivar mixture was related to the parasitism rate, supporting associational resistance as a principal strategy for aphid control (Koricheva & Hayes 2018).

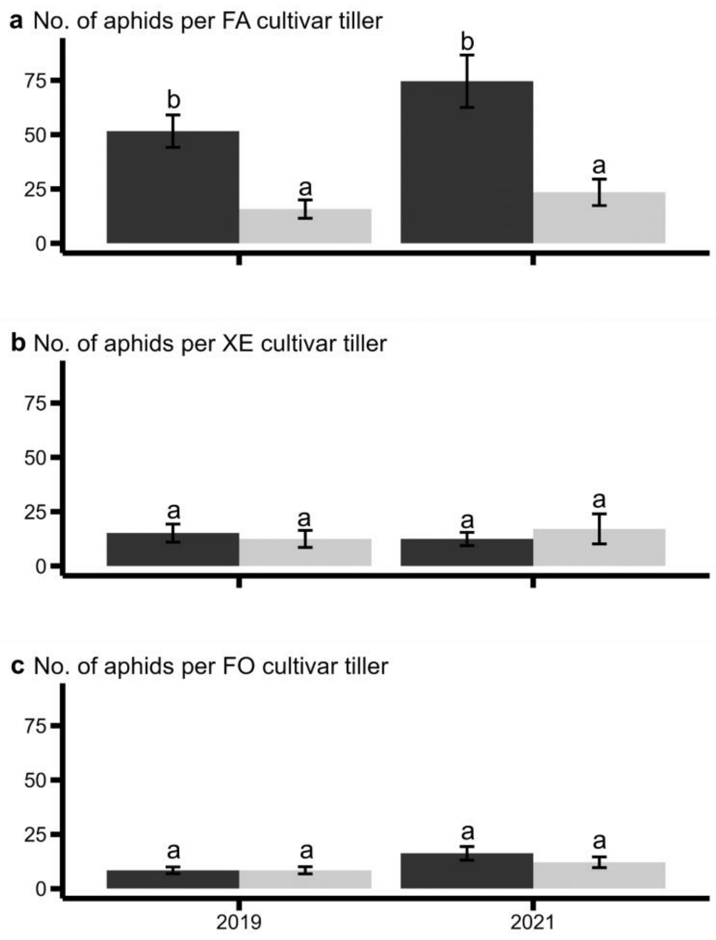

Figure 5 Number of aphids per tiller (mean ± SE) at peak time on individual plants grown in monoculture (black bars) and cultivar mixture (grey bars) in 2019 and 2021. a. Florence-Aurora cultivar, b. Xeixa cultivar, c. Forment cultivar. Different letters above bars indicate statistically significant differences according to Tukey-adjusted pairwise EMMS comparisons (P<0.05).

3.4. Crop yield service

This paper explores the performance of modern and traditional wheat cultivar monocultures and wheat cultivar mixtures sown under contrasting management practices in field conditions. Our goal was to design practical and appealing polycultures for farmers to adopt in real farming. To achieve this, we assessed crop yield, as it is the most relevant variable for farmers when implementing diversity-related strategies. Moreover, we combined wheat cultivars with similar crop husbandry requirements and commercial purposes. This concern has been widely overlooked in the literature thus far (Di Bene et al. 2022).

Similar to wheat establishment and wheat cover, crop yield was significantly lower in 2021 ($F_{1,63} = 11.24$, $P < 0.01$) most likely caused by differences in rainfall, which is known to be one of the main drivers of yield in the Mediterranean region (Basso et al. 2012; Gandía et al. 2021). Fertilization emerged as the primary factor affecting wheat yield ($F_{1,63} = 23.61$, $P < 0.001$), resulting in a 9% difference in 2019 and a 29% difference in 2021 compared to nonfertilized plots. Conventional tillage tended to enhance wheat yield in both years, with a significant effect in 2021 ($F_{1,63} = 9.31$, $P < 0.05$). Predictably, wheat cover had a positive correlation with yield ($F_{1,63} = 17.84$, $P < 0.001$). In general, crop types had similar yields ($F_{3,61} = 7.80$, $P = 0.13$). However, traditional cultivar monocultures, XE and FO, displayed significantly higher yields in nonfertilized and reduced tillage conditions than the modern cultivar FA. Furthermore, FA and MIX yield was conditioned by tillage and fertilization, resulting in lower yield values under nonfertilized and reduced tillage plots (Figure 6).

Soil management practices significantly affected crop yield. Supporting prior findings, fertilization was a limiting factor for optimal wheat yield in Mediterranean organic fields, perhaps because of the low wheat nitrogen use efficiency in calcareous soils (Baldivieso-Freitas et al. 2018; Diacono et al. 2019; Tamburini et al. 2016). Additionally, reduced tillage led to a decrease in crop yield, likely due to a temporary delay in nitrogen mineralization, creating a nitrogen deficit in early spring that impacts yield (Mäder & Berner 2012).

Modern Florence-Aurora cultivar monoculture and cultivar mixture yield depended on tillage and fertilization conditions. The highest yields occurred under conventional practice scenarios. In contrast, traditional cultivar monocultures were unaffected by soil management practices. These differences can be explained by the great dependence of modern cultivars on external inputs and their poor performance against weed infestation and under nitrogen shortage, which are common in Mediterranean organic crops (Baldivieso-Freitas et al. 2018; Lazzaro et al. 2018; Reiss & Drinkwater 2018; Worthington et al. 2013).

Nevertheless, the LER analysis suggests neutral or limited advantages of the cultivar mixture in terms of yield. LER values varied depending on tillage practices and fertilization conditions, ranging from 0.90 in 2021 under conventional tillage practices without fertilization to 1.19 in 2021 under reduced tillage practices with fertilization. These findings are consistent with previous studies that did not show yield improvement on wheat cultivar mixtures over monocultures

(Lazzaro et al. 2018; Mansion-Vaquié et al. 2019). It is worth to point out that under reduced tillage and no fertilization, the LER was larger than 1.0 in both years (1.03 and 1.01), hinting that traditional cultivars may offer functions such as weed suppression or soil-nitrogen shortage tolerance that enable higher yield stability (Reiss & Drinkwater 2018).

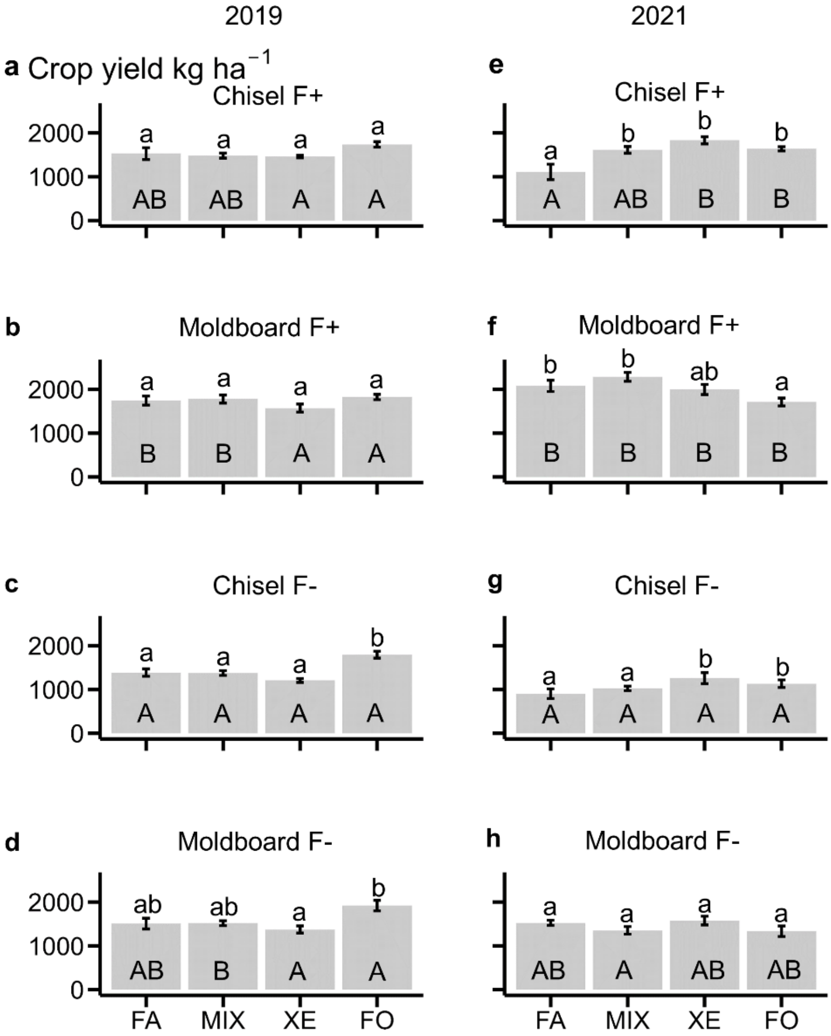

Figure 6 Wheat yield (mean ± SE) of the different crop types (Florence-Aurora (FA), Xeixa (XE), and Forment (FO) monocultures and their mixture (MIX)) in 2019 (a-d) and 2021 (e-h). a, e: Crop yield under reduced tillage practices and with fertilization. b, f: Crop yield under conventional tillage practices and with fertilization. c, g: Crop yield under reduced tillage practices and without fertilization. d, h: Crop yield under conventional tillage practices and without fertilization. Lowercase letters denote significant differences within rows according to Tukey-adjusted pairwise EMMS comparisons (P < 0.05) within treatments, separately for each year. Capital letters indicate significant differences within columns (P < 0.05) for each crop type across the four tillage and fertilization treatments. Each year was analyzed independently.

4. Conclusions

The wheat cultivar mixture showed several advantages compared to the modern variety monoculture because of the functions offered by the traditional cultivars. In this regard, the cultivar mixture provided aphid control through associational resistance and weed suppression capability while preserving weed diversity. Furthermore, for the first time, we provide experimental support for changes in the performance of a wheat cultivar mixture caused by management practices. Therefore, this study emphasizes the importance of field experiments to assess the influence of agronomical factors present in real farming scenarios that may interfere with the cereal mixture functionality.

Acknowledgements

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Supplementary material

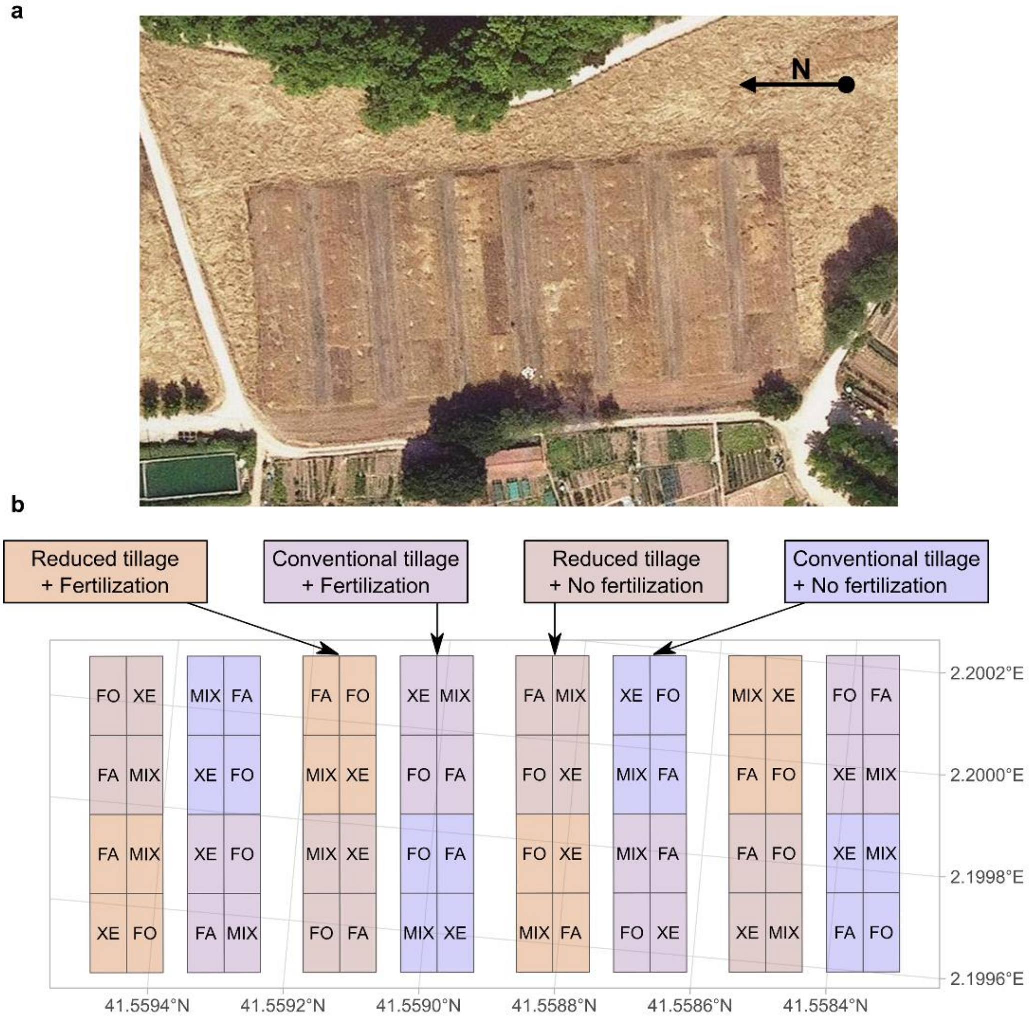

Figure SM 1 a. Orthographic photograph of the commercial field before harvest time (late-June 2021). b. Schematic representation of the strip-split block experimental design with four replicates, showing the distribution of tillage and fertilization treatments, along with the 64 subplots designated for the crop type treatment (FA, XE, FO and MIX: Florence-Aurora, Xeixa, Forment and Mixture cultivars, respectively).

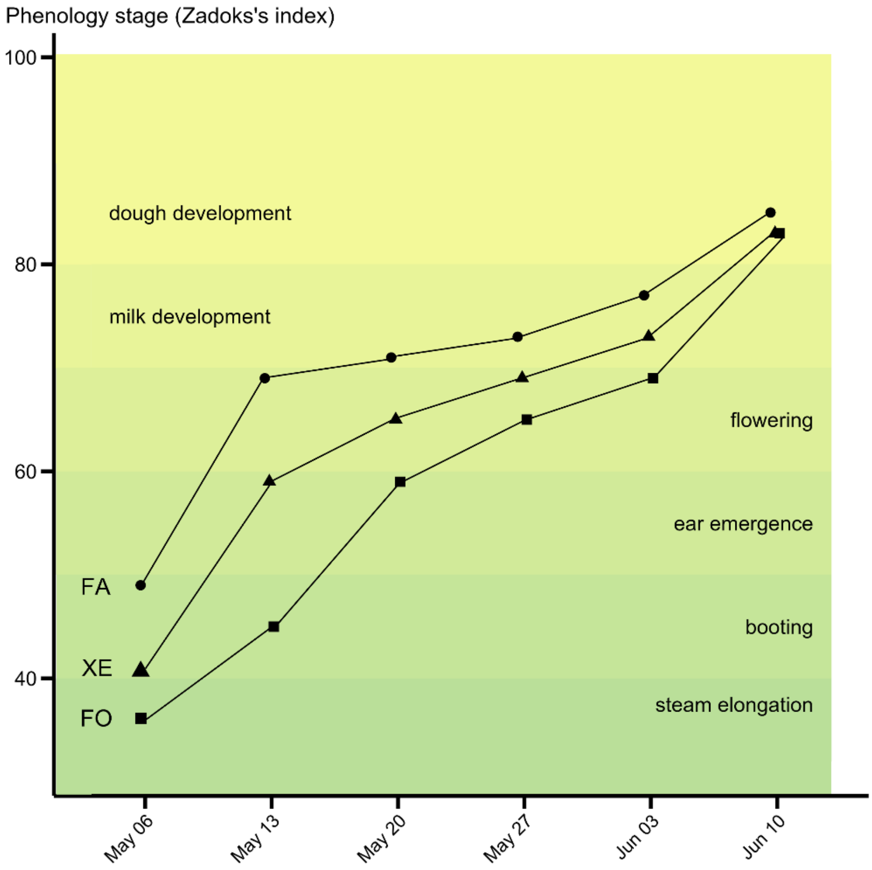

Figure SM 2 Wheat cultivar phenological development (median with 95% CI) from May to June 2019. Text within the colored area of the plot corresponds to phenology stages expressed in the Zadoks scale. Wheat cultivars: Florence-Aurora (FA), Xeixa (XE), and Forment (FO).

Capítol 2

Associating cultivars or species with complementary traits is key for enhancing aphid control through bottom-up effects

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Associating cultivars or species with complementary traits is key for enhancing aphid control through bottom-up effects

Resum

L'agricultura ecològica promou estratègies de diversificació per millorar entre d'altres, el control de plagues en els cultius. Tanmateix, els efectes dels policultius de cereal en el control de les poblacions de pugons no és encara clar, sovint perquè no es tenen en compte els trets funcionals de les espècies i varietats associades. El nostre estudi té l'objectiu d'avaluar el control de pugons, així com els mecanismes *bottom-up* i *top-down* relacionats i la producció de cultius de blat amb diferents trets funcionals (susceptibilitat als pugons, alçada, fenologia i contingut de nitrogen en gra). Es van establir deu tractaments replicats en cinc camps ecològics: tres monocultius de blat (Florence-Aurora (FA), Montcada (MO) i Forment (FO)), una barreja de varietats amb trets similars (FAMO) i una barreja de veritats amb trets diferents (FAFO), cadascun duplicat sense i amb una cobertura vegetal de melgó (*Medicago polymorpha*). Es va analitzar l'abundància de pugons, el nombre de pugons per fillol, la taxa de parasitisme, l'abundància d'artròpodes depredadors i la producció.

Els resultats mostren que FAFO i la coberta de melgó van reduir significativament l'abundància de pugons i el nombre de pugons per fillol en les varietats de Florence-Aurora, mentre que FAMO no va disminuir significativament la població de pugons. Per tant, els beneficis dels policultius en el control de la població de pugons depenen de la identitat de les varietats associades, sent més avantatjós l'associació de varietats amb trets funcionals complementaris. Tanmateix, la combinació de FAFO amb la coberta de melgó no resultà beneficiosa, probablement a causa de la redundància entre les funcions de la varietat resistent, Forment, i el melgó. D'altra banda, els diversos cultius de blat van presentar una abundància de predadors i una taxa de parasitisme similar, així doncs, la reducció de la població de pugons deriva de mecanismes *bottom-up*. Finalment, la producció de blat va ser comparable entre tractaments, excepte en la temporada 2021, on FA va produir significativament menys.

1. Introduction

In recent decades, cereal agricultural systems have experienced a notable trend towards simplification with the adoption of monoculture systems and the gradual replacement of traditional cereal cultivars with a limited selection of modern cultivars, which currently account for the 97 % of sown cereals (Aragon et al. 2009; Tilman et al. 2011). These modern cultivars are highly reliant on agrochemicals and machinery, and their performance can be negatively affected by drought, high temperatures, and pest infestation, which are common in the Mediterranean basin area (Bonnet et al. 2021; Ficiciyan et al. 2018). Furthermore, the implementation of modern cultivar monocultures led to a significant decrease in diversity, including both the genotypic diversity of crops and the diversity of beneficial arthropods (Dainese et al. 2019; Ficiciyan et al. 2018). This simplification of the system can cause reduced pest resistance and decreasing yield, among other issues (Hatt & Döring 2023; Reiss & Drinkwater 2018). To counter this trend, organic farming advocates for diversity-based strategies as a method for increasing in-field complexity and enhancing system functionality (Barot et al. 2017; Gaba et al. 2015; Hatt & Döring 2023; Wezel et al. 2014).

At the field scale, agrobiodiversity can be classified into two components: planned diversity, which refers to the diversity of cash, forage, or cover crops that are intentionally incorporated into the field, and associated diversity, that includes all the microorganisms, arthropods, and weeds that interact in the system (Altieri & Rogé 2009). Planned diversity can be either intraspecific or genotypic (intracropping) or interspecific (intercropping). Genotypic diversity is concerned to the association of cultivars, whereas interspecific diversity involves the combination of different species, such as the association of a cash crop with a cover crop, also referred to as undersowing (Andow 1991).

Polycultures increase field complexity and ecological interactions, which may enhance the provision of ecological functions such as aphid population control and yield stability (Borg et al. 2018; Costanzo & Bàrberi 2014; Gurr et al. 2017; Malézieux et al. 2009). Aphids are phytophagous insects considered potential pests in cereal crops. In wheat cultivation, aphids can reduce production both directly, by extracting nutrients from the host plant, and indirectly, by serving as vectors for transmitting viruses like the ones causing dwarfism and stunting (such as BYDV, Barley Yellow Dwarf Virus), among others (Dedryver et al. 2010). Moreover, they are an interesting model organism for the study of insect-plant interactions (Rodriguez-Saona & Stelinsky 2009).

The potential of cereal polycultures for aphid control relies primarily on two nonexclusive sets of ecological processes: bottom-up and top-down effects. In bottom-up control, aphid population regulation is driven by associational resistance, which is the favorable association between cultivars and nonhost cultivars or crops (Barbosa et al. 2009). Associational resistance supports the disruptive crop hypothesis (Tahvanainen & Root 1972), which suggests that potential pests are less likely to find their target host when it is masked by less preferred cultivars or nonhost species. For instance, Shoffner and Tooker (2013) conducted a laboratory experiment that revealed associational resistance when mixing six-line wheat cultivars with varying levels of aphid resistance.

Regarding top-down control, it is impelled by associated diversity, such as predatory arthropods and parasitoid wasps (Gurr et al. 2017). Top-down effects are based on the natural enemies' hypothesis, which states that planned diversification creates conditions that promote the abundance and richness of beneficial arthropods (Altieri & Rogé 2009; Gurr et al. 2017; Letourneau et al. 2011). For example, early research showed a positive effect of barley mixture on the abundance of ladybirds (Ninkovic et al. 2011). Some other studies have shown that legume undersowing can provide shelter, varied microclimate conditions, and additional resources, for instance, a broader range and greater abundance of alternative prey, that increases the population of aphid predators such as ladybirds or spiders (Dassou & Tixier 2016; Letourneau et al. 2011).

However, the majority of studies on the functionality of polycultures were conducted in controlled environments, overlooking the complexity found in actual field conditions. Furthermore, prior field research has reported inconsistent evidence regarding the advantages of cereal polycultures for aphid control (Dahlin et al. 2018; Mansion-Vaquié et al. 2019; Ninkovic et al. 2002). This discrepancy can be attributed to the fact that many studies did not take into consideration the functional traits when combining cereal varieties or intercropping, potentially leading to functional redundancies or negative interactions, such as competition. Consequently, increasing the complexity of the cropping system did not necessarily translate into an enhanced system's functionality (Barot et al. 2017; Brook et al. 2020; Gaba et al. 2015; Ninkovic et al. 2016).

For this study, we established various wheat polycultures to evaluate their functionality under real farming conditions. Functionality was estimated based on the capacity to provide aphid control. To achieve this, we combined the dominant wheat cultivar in the study area, Florence-Aurora, with one cultivar that shared similar functional traits and with another cultivar that had distinct functional traits. The traits considered to be relevant for aphid control were height, odor profile, and nitrogen content (Barot et al. 2017; Nowak & Komor 2010; Webster 2012). Wheat intercropping was established by associating wheat crops with legume undersowing. This decision was driven by the increasing adoption of cereal and legume intercropping in organic farming due to the well-documented benefits of legume plants in enriching soil with nitrogen, and thus increasing cereal yields (Wezel et al. 2014). For the legume undersowing we sowed burclover (*Medicago polymorpha* L.) because it is a common herbaceous plant in Mediterranean arable lands. Finally, we examined whether stacking (combining genotypic and interspecific diversity practices) may lead to a potential synergy boosting aphid population control by providing complementary strategies to the cropping system (Hatt & Döring 2023).

Therefore, the aim of this study is to assess the effect of contrasted genotypic diversity, interspecific diversity, and their stacking on aphids' populations and their control agents in Mediterranean organic winter wheat crops. We explored the ecological processes underlying aphid control, specifically the associational resistance and the influence of polycultures on parasitism rate and predator's abundance. Finally, we analyzed wheat yield to assess the viability of the wheat polycultures to be used by farmers. We hypothesized that (i) cultivar mixtures with complementary aphid-resistance traits reduce aphid abundance by associational resistance, (ii) legume cover crop increases parasitism rate, ground- and foliage-dwelling arthropod predators' abundance, and (iii) stacking genotypic and interspecific diversity outperforms monocultures, intracropping and monocultures associated with legume undersowing, and (iv) polyculture provides increased yield compared to monocultures.

2. Methodology

2.1. Study sites

This study was performed during two cropping seasons (2019-2020 and 2020-2021) in the rural area of Gallecs, which is a periurban agricultural area of 755 ha located 15 km north of Barcelona (41º33'31.9" N 2º11'59.5" E, Catalonia, Northeast Spain). The area has a Mediterranean climate with dry and hot summers and mild winters, with a mean annual temperature of 14.6 ºC and 629.2 mm mean annual rainfall. During the two cropping seasons, from December to June, the mean temperature and accumulated precipitation were 13.9 ºC and 532.3 mm in 2019-2020 and 13.2 ºC and 83.8 mm in 2020-2021, respectively. At the beginning of the experiment, we analyzed the soil properties of the five experimental fields. The average soil organic matter was 1.7 ± 0.07 %. and nitrogen content was 0.17 ± 0.02%. The soil was a slightly alkaline (pH 8.6) loamy clay.

2.2. Winter wheat cultivars and legume undersowing

The winter wheat cultivars used in the experiment were selected through collaborative discussions with local farmers and flour makers. We considered three common cultivars with similar agronomic needs and commercial purposes to assure the viability of the cultivar mixtures in a real farming context.

The three wheat cultivars chosen were Florence-Aurora (*Triticum aestivum* L. subsp. *aestivum*), which flour is considered excellent for making bread, and two traditional cultivars: Montcada (*Triticum aestivum* L. subsp. *aestivum*), which is another good variety for making bread, and Forment (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.), which confers valuable aromatic properties. The three cultivars have a similar harvest time, and their flour is already mixed for bread manufacturing in mills.

Florence-Aurora and Montcada present similar functional traits related to aphid control but distinct from Forment's. Florence-Aurora and Montcada reach 70 cm at harvest time, whereas Forment reaches nearly 180 cm (Serra-Gironella & Álvaro 2017). Aerial architecture can affect aphids' mobility (Barot et al., 2017). Moreover, Florence-Aurora and Montcada have similar odor profiles, which differ from Forment's (Tous-Fandos et al., 2023). Wheat cultivar odor profile is the composition of volatiles compounds (VOCs) released by the plants. It plays an important role in aphid host localization (Webster, 2012). Finally, we analyzed the tissue nitrogen content by

Dumas's combustion of ten 1-month-old individuals grown in pure stands under greenhouse conditions (18–22 °C with a light regime of L16:D8 h), as nitrogen content is a host quality trait that conditions aphid host selection (Nowak & Komor 2010). Florence-Aurora's total nitrogen content was 52.8 ± 0.7 g N kg^{-1}, Montcada was 52.2 ± 0.5 g N kg^{-1} and Forment was 50.9 ± 0.5 g N kg^{-1}.

We selected burclover (*Medicago polymorpha* L.), a fast-growing sprawling winter annual with weak stems reaching a length of 10–50 cm, as an undersowing crop because it is an N-fixing legume common in the Mediterranean region.

2.3. Field experimental design

The experimental design consisted of ten treatments laid out randomly on ten experimental plots of 40 m by 18 m established in a commercial field. There were five wheat plot types: three monocultures of Florence-Aurora (FA), Montcada (MO), and Forment (FO), and two cultivar mixtures of Florence-Aurora and Montcada (FAMO) and Florence-Aurora and Forment (FAFO). Each one of these five wheat plot types was cultivated with the presence (M +) or absence (M -) of a burclover legume undersowing, totaling ten treatments. The experimental design was replicated in five commercial fields, that were organically managed since 2006, during two consecutive years. To reduce variation between fields, we selected five fields that were similar in size, soil, and surrounding landscape composition, as well as previous agricultural management practices (Chamorro et al. 2017) (Figure 1).

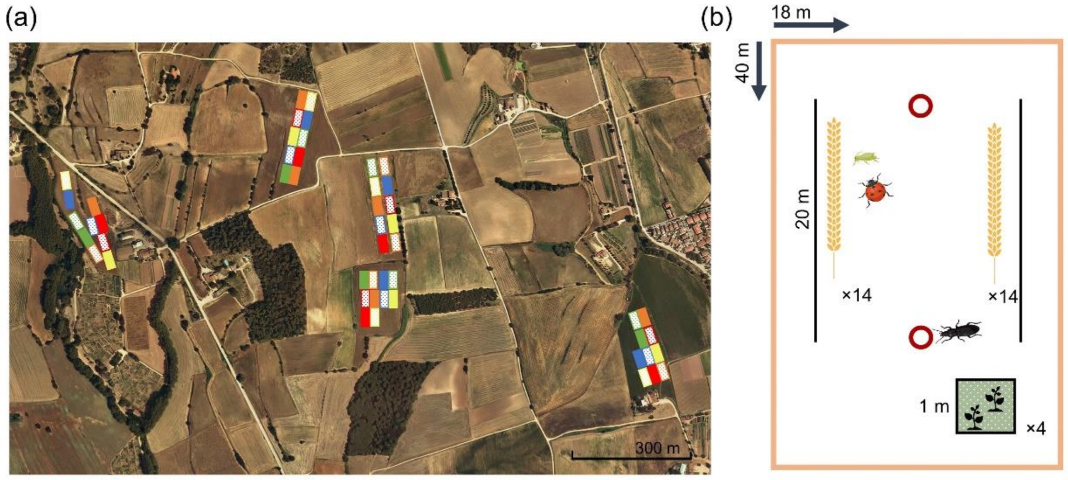


Figure 1 Illustration of the study area and sampling design (a) Map of the five fields used in this experiment, with colored rectangles denoting the experimental plots. Each color represented a wheat treatment. Florence-Aurora monoculture: blue., Montcada monoculture: green., Forment monoculture: red., Florence-Aurora and Montcada mixture: yellow., and Florence-Aurora and Forment mixture: orange. Pointed pattern rectangles represented plots with burclover undersowing. (b) A schematic of the experimental plot design, showing the two transects for

sampling aphids, parasitoids, and foliage-dwelling predators (black lines), the two pitfall traps for sampling ground-dwelling predators (brown cercles), and the squares for burclover cover samplings.

In 2019 and 2020, we applied 30 t ha^{-1} of farmyard composted manure and employed chisel tillage followed by a rotary harrow for seedbed preparation before sowing. Winter wheat cultivars and burclover were sown with an interval of less than 5 days between them (sowing dates: 19–21 November 2019 and 01–03 December 2020) at seed densities of 180 kg ha^{-1} and 2.6 kg ha^{-1} respectively. Cultivar mixtures comprised 50 % Florence-Aurora and 50 % Montcada or Forment, with prior mixing of the seeds to ensure homogeneity.

2.4. Field Sampling

2.4.1. Aphids and predators

Cereal aphid and predators' samplings were carried out every three weeks from early March to late May during the two cropping seasons. We counted aphids and mummies (parasitized aphids) on 28 wheat tillers evenly distributed along two parallel 20 m transects (14 tillers per transect) situated 5 m apart from the border in each plot. In cultivar mixtures plots, we distinguished wheat cultivars and aphids were counted separately on each cultivar (14 tillers per cultivar, totaling 28 tillers per plot). Aphid species were identified in the field using magnifiers and visual identification keys for the most common species. We collected mummies containing parasitoids that had not yet emerged and kept them separately in individual vials in the lab at room temperature (20–24 °C, with no control over humidity) until the parasitoids emerged. Both the parasitoid adult and the aphid mummies were then preserved in 70% ethanol. We identified hatched primary and secondary parasitoids at the genus level.

The total number of aphids counted in each plot throughout the entire sampling season served as a proxy for measuring aphid abundance. It expressed overall aphid pressure. At aphid peak time, in late April 2020 and early May 2021, we measured the number of aphids per tiller. The number of aphids per tiller was calculated as the mean number of aphids per single cultivar tiller per plot. Every cultivar was analyzed separately. Hence, it enabled us to compare the aphid infestation on each cultivar when grown in different conditions: monoculture, cultivar mixture, and with or without burclover undersowing. We used the number of aphids per tiller as an indicator for the disruptive crop hypothesis from bottom-up aphid control. In contrast, to test the natural enemies' hypothesis, we used parasitism rate, and predators' abundance distinguishing between foliage-dwelling and ground-dwelling predators. The parasitism rate was calculated by dividing the total number of mummies by the sum of non-mummified and mummified aphids per plot.

We visually recorded the abundance of foliage-dwelling predators along the 20 m transects in 5 min. The walking speed was 2 m min^{-1}, and we counted all predators within 1 m on either side of each transect. To improve our observations, we carefully examined the spikes and tillers of the wheat, and we adjusted our view angle to ensure that every area was visible. The target groups were ladybirds (Coleoptera: Coccinellidae) in the adult and larval stages, hoverflies (Diptera: Syrphidae) in the larval stage, adult soldier beetles (Coleoptera: Cantharidae) and adult spiders (Araneae). The recording of foliage-dwelling predators was conducted from 9 a.m. to 5 p.m. in fair weather conditions.

We installed two pitfall traps along the central line of each plot, 9 m from the lateral edges and 10 m from either end, to assess ground-dwelling predators. Each trap comprised a plastic cup (Ø 65 mm, 100 mm deep) filled with propylene glycol (diluted at 30% in distilled water) as a preservative solution. We covered the pitfall traps with a floor tile to limit the bycatch of small vertebrates or the interference with birds or rain throughout the open period. The traps were installed for periods of 48 h each, three times in 2020 and four times in 2021. We stored the collected samples in 70% ethanol. The identification of ground-dwelling predators was at the order level (i.e; Dermaptera, Opiliones), at the family level in rove beetles (Coleoptera: Staphylinidae), and at the genera level in ground beetles (Coleoptera: Carabidae) and spiders (Araneae). Ground beetle and spider richness were analyzed.

2.4.2. Burclover ground cover

At aphid peak time, we analyzed the relation between the ground cover of burclover and aphid abundance in plots with burclover undersowing to deepen the effect of nonhost cover on aphid control. The sampling was performed every three weeks from March to June in the 2020 season. Well-trained samplers visually estimated the percentage of burclover cover in four random 1 m^2 quadrats per plot.

2.4.3. Crop yield

The total dry grain weight per plot (kg ha^{-1}) served as an estimate of crop yield. The plots were harvested at the end of June (20 – 23 of June both years) by a commercial harvester, and the grain was weighed on-site with a crane scale. Then, 800-1000 g grain aliquots were saved, separated from impurities in the laboratory, and dried (48 h oven-dried at 60 ºC) to extrapolate grain dry weight without impurities.

Our sampling had two limitations: The 2020 season started one month later than 2021 due to COVID-19 pandemic restrictions. Consequently, only three out of four samplings per year were evaluated. Second, burclover did not grow well in 2021. The poor establishment of burclover prevented the inclusion of this factor in the statistical analysis of the data collected in 2021.

2.4.4. Statistical analysis

All statistical analyses were conducted using R, version 4.1.1 (R Development Core Team 2018). The measured variables of aphid abundance, the number of aphids per tiller, total parasitism rate, total abundance of ground-dwelling predators and total abundance of foliage-dwelling predators were analyzed with generalized linear mixed effects models (GLMM). The crop yield model was assessed with a linear mixed effects model (LMM). The total number of ground- and foliage-dwelling predatory individuals was insufficient to conduct a rigorous GLMM analysis for each taxonomic group. For modeling, we used the *glmmTMB* function from the *glmmTMB* package (Brooks et al. 2017).

Five models were fitted with three fixed explanatory variables: wheat treatment (W) (categorical with five levels: FA, MO, FO, FAMO, FAFO), burclover undersowing (M) (categorical with two levels: M+, M-), year (Y) (categorical with two levels: 2020, 2021), and their interactions. The field factor was included as a random effect variable. Model 1 contemplated three-way interaction (W × M × Y), Model 2 did not consider any interactions, Model 3 accounted for the two-way interaction between wheat and burclover undersowing (W × M), Model 4 contemplated the two-way interaction between wheat and year (W × Y), and Model 5 considered the two-way interaction between burclover undersowing and year (M × Y). The significance of fixed effect factors and their interactions was determined with an F test with Kenward-Roger approximation for LMMs or a likelihood ratio test (LRT) for GLMMs. Pairwise comparisons were carried out using Tukey-adjusted estimated marginal means from the *emmeans* package (Lenth et al. 2019). The best model for each response variable was considered according to the Akaike information criterion corrected for small sample sizes (AICc). The simulateResiduals function from the *DHARMa* package was utilized to examine normality, homoscedasticity and residual distribution (Hartig 2019).

Aphid abundance was analyzed with a negative binomial. For the statistical evaluation of the number of aphids per tiller, each cultivar was analyzed independently. Hence, we categorized the wheat treatment factor into two distinct levels: monoculture (FA, MO, or FO) and crop mixture (FAMO or FAFO). To equalize the sowing ratios between monocultures and mixtures, the total number of aphids per single wheat tiller cultivar in mixture plots was doubled. Then, the mean number of aphids per tiller was fitted to a negative binomial. The total parasitism rate was analyzed with a binomial. The ground- and foliage-dwelling predator abundance were fitted to a negative binomial.

We employed a linear regression to examine the relationship between aphid abundance and burclover cover.

3. Results

3.1. Aphid abundance

We recorded a total of 27,417 aphids in five fields during the two sample seasons belonging to 7 species, the most abundant being *Sitobion avenae* (Fabricius, 1775), which represented the 78% of the total aphid abundance (see Appendix S1: Table S1 for a complete list of all aphid species found). Given the predominance of *S. avenae* throughout all treatments, we refrained from conducting species-level analyses for aphid variables.

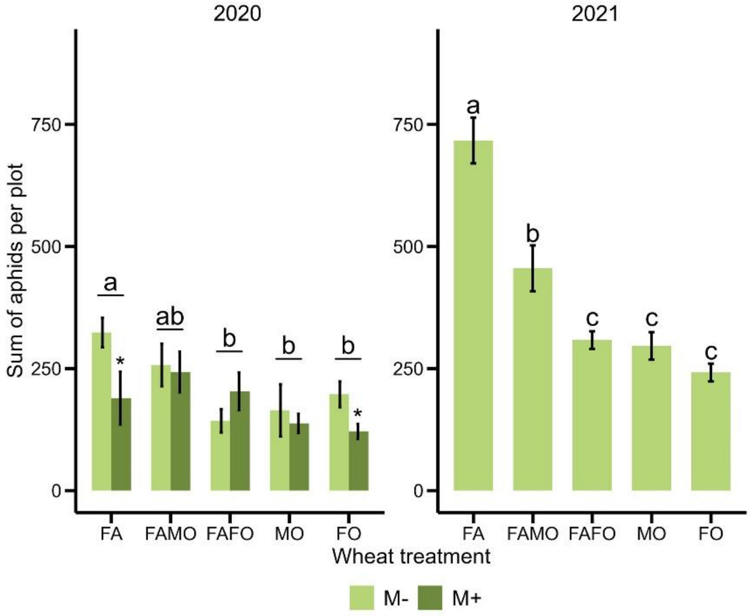


Figure 2 Aphid abundance (mean ± SE) over the 2020 and 2021 seasons on 5 wheat treatments: Florence-Aurora monoculture (FA), Florence-Aurora and Montcada mixture (FAMO), Florence-Aurora and Forment mixture (FAFO), Montcada (MO) and Forment (FO) monoculture with (M+) or without (M-) burclover undersowing. Burclover treatment was dismissed in 2021 for its poor establishment. Letters indicate significant differences within wheat treatment and asterisks within burclover treatment according to Tukey-adjusted pairwise comparisons ($P < 0.05$). Every year was analyzed independently.

Table 1 Results of the linear mixed effects model (LMM) and generalized linear mixed effects models (GLMM) selection relating three fixed covariables: wheat treatment (W), burclover undersowing (M), year (Y) and their interactions. The significance of fixed effect factors and their interactions was determined with an F test with Kenward-Roger approximation for LMMs or a likelihood ratio test (LRT) for GLMM. Field factor was included as a random effect variable. Only the best model is presented for each response variable. Model 1= W + M+ Y + W × M × Y, Model 2= W + M+ Y, Model 3= W + M+ Y + W × M, Model 4= W + M+ Y + W × Y, Model 5= W + M + Y + M× Y.

Response variable	Model	Wheat (W)	Burclover (M)	Year (Y)	W×M	W×Y	M×Y	W×M×Y
Aphid abundance	Model 4	$\chi_{4,7} = 125.2$	$\chi_{1,10} = 5.1$	$\chi_{1,10} = 123.0$	-	$\chi_{4,7} = 17.2$	-	-
		$P < 0.001$	$P < 0.04$	$P < 0.001$	-	$P < 0.007$	-	-
Number of aphids per Florence-Aurora tiller	Model 1	$\chi_{2,10} = 14.4$	$\chi_{1,3} = 0.01$	$\chi_{1,11} = 11.5$	$\chi_{2,10} = 13.4$	$\chi_{2,10} = 15.0$	$\chi_{1,11} = 0.1$	$\chi_{2,10} = 10.7$
		$P < 0.001$	$P = 0.89$	$P < 0.001$	$P < 0.005$	$P < 0.001$	$P = 0.76$	$P < 0.008$
Number of aphids per Montcada tiller	Model 2	$\chi_{1,3} = 0.8$	$\chi_{1,4} = 1.5$	$\chi_{1,3} = 0.01$	-	-	-	-
		$P = 0.36$	$P = 0.21$	$P = 0.92$	-	-	-	-
Number of aphids per Forment tiller	Model 5	$\chi_{1,4} = 0.3$	$\chi_{1,11} = 0.61$	$\chi_{1,4} = 9.7$	-	-	$\chi_{1,4} = 4.6$	-
		$P = 0.60$	$P = 0.60$	$P < 0.009$	-	-	$P < 0.04$	-
Parasitism rate	Model 2	$\chi_{4,8} = 17.1$	$\chi_{1,6} = 0.60$	$\chi_{1,11} = 33.8$	-	-	-	-
		$P < 0.004$	$P = 0.43$	$P < 0.001$	-	-	-	-
Total ground-dwelling predators	Model 2	$\chi_{4,3} = 2.3$	$\chi_{1,7} = 0.6$	$\chi_{1,6} = 129.7$	-	-	-	-
		$P = 0.68$	$P = 0.84$	$P < 0.001$	-	-	-	-
Total foliage-dwelling predators	Model 5	$\chi_{4,4} = 11.8$	$\chi_{1,7} = 5.3$	$\chi_{1,7} = 37.8$	-	-	$\chi_{1,7} = 2.2$	-
		$P < 0.02$	$P < 0.04$	$P < 0.001$	-	-	$P = 0.14$	-
Crop yield	Model 4	$F_{4,7} = 3.9$	$F_{1,10} = 0.6$	$F_{1,10} = 0.003$	-	$F_{4,7} = 22.8$	-	-
		$P = 0.45$	$P = 0.82$	$P = 0.99$	-	$P < 0.001$	-	-

The analysis of aphid abundance revealed significant effects of year, wheat and burclover factors, as well as W×Y interaction (Table 1). Aphids were more abundant in 2021 ($P = 0.007$). Over both sampling seasons, FA plots supported higher aphid abundance compared to FO and MO ($P < 0.001$). Cultivar mixtures displayed varied outcomes depending on identity and year. On the one hand, FAFO exhibited similar aphid abundance to FO, and significantly lower than FA ($P = 0.005$), consistently across years. On the other hand, FAMO, FA and MO presented comparable aphid abundances in 2020. However, in 2021, MO had a significantly lower abundance, FAMO was intermediate, and FA had a significantly higher one ($P = 0.03$). Aphid abundance was higher in FAMO than in FAFO plots, but this difference was significant only in 2021 ($P = 0.04$). Aphid abundance was lower in plots with burclover undersowing, except in FAFO plots. The Burclover effect was statistically significant in FA and FO plots ($P = 0.03$) (Figure 2).

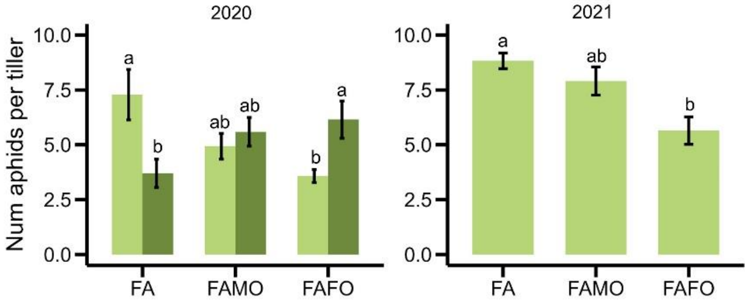
3.2. Number of aphids per tiller

The analysis of the number of aphids per tiller of FA cultivar presented a significant effect of wheat treatment and W × M interaction (Table 1). Both years, the number of aphids per tiller tended to be reduced in FA grown in mixtures, but only those in FAFO presented a significantly lower value ($P = 0.02$). Additionally, the presence of burclover undersowing had divergent effects. It decreased the number of aphids per tiller in FA plots ($P = 0.03$) but increased it in FAFO ($P = 0.04$) (Figure 3a). The number of aphids per tiller of MO and FO cultivars was not influenced by any studied treatment (Figure 3b–3c).

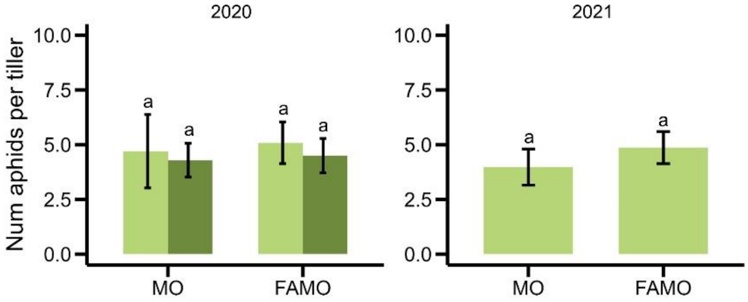
3.3. Burclover ground cover and aphid abundance relationship

When examining the overall relationship between burclover cover and aphid abundance, we found a significantly negative association between estimated burclover ground cover percentage and aphid abundance ($P < 0.001$, $r^2 = 0.36$) (Figure 4a). This pattern was consistent when individual wheat treatments were analyzed separately, with statistical significance observed in FA and FO treatments ($P = 0.02$, $r^2 = 0.41$; $P < 0.001$, $r^2 = 0.73$, respectively) (Figure 4b). Nevertheless, there was no correlation between aphid abundance and burclover cover in FAFO plots (Figure 4c).

(a) Florence-Aurora



(b) Montcada



(c) Forment

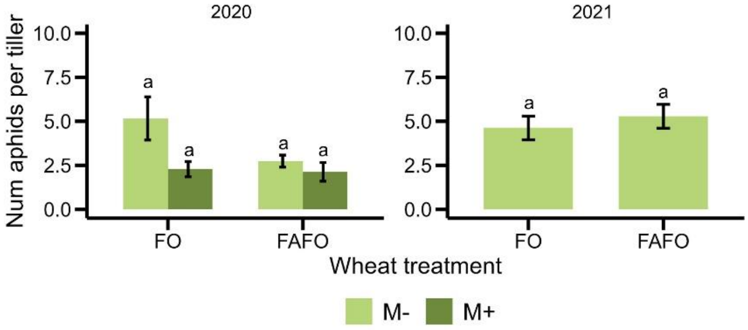


Figure 3 Number of aphids per tiller (mean ± SE) at aphid peak time (late April 2020, early May 2021) in each wheat cultivar (a) Florence-Aurora cultivar grown in monoculture (FA), mixed with Montcada (FAMO), and mixed with Forment (FAFO) (b) Montcada cultivar grown in monoculture (MO) or mixed with Florence-Aurora (FAMO) (c) Forment cultivar grown in monoculture (FO) or mixed with Florence-Aurora (FAFO). All crop types intercropped with (M+) or without (M-) burclover undersowing. Burclover treatment was dismissed in 2021 for its poor establishment. Letters indicate significant differences within wheat treatment according to Tukey-adjusted pairwise comparisons ($P < 0.05$). Every year was analyzed independently.

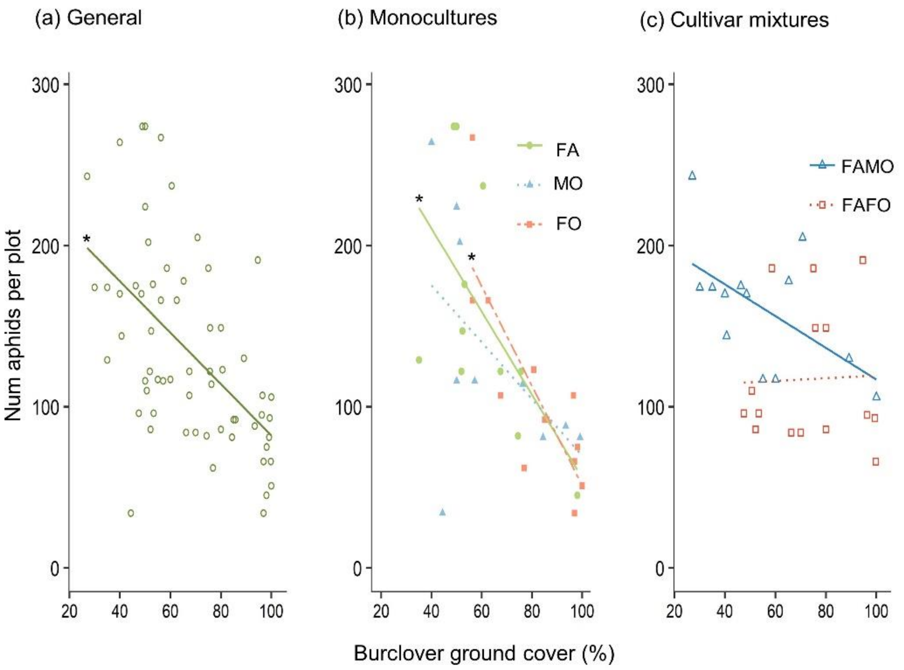


Figure 4 Regression analyses relating burclover ground cover and number of aphids at peak time (late April 2020, early May 2021). First-order polynomials best described the relation between burclover ground cover (%) and aphid abundance in all the analyses. (a) General analysis including all plots (b) Monocultures: Florence-Aurora, Montcada (MO) and Forment (FO) (c) Cultivar mixtures: Florence-Aurora and Montcada mixture (FAMO), Florence-Aurora and Forment mixture (FAFO). Asterisks indicates significant relationship analyzed by linear regression (P < 0.05).

3.4. Parasitoid and aphid predator abundance

We collected 2276 mummies (open and closed) during the two sample seasons. We identified nine wasp genera, with *Aphidius* dominating as the main primary parasitoid genus (60%) and *Asaphes* as secondary parasitoids (10%) (Further details Appendix S1: Table S1). The overall parasitism rate was 11.5 % in 2020 and decreased to 5.1 % in 2021 (P < 0.001).

Wheat treatment significantly affected parasitism rates in both years (Table 1). Nonetheless, the results were discordant. In 2020, FO plots exhibited a lower parasitism rate (7.2 % ± 1.3) when compared to FA (11.4 % ± 2.9) and FAMO plots (12.1 % ± 2.7). In 2021, however, FAMO had the highest parasitism rate (5.4 ± 0.7), and FA showed the lowest rate (3.9 % ± 0.7). Burclover undersowing did not influence the parasitism rate (Table 1).

We recorded a total of 2050 foliage-dwelling predatory arthropods from the surveys. The most abundant group was ladybirds, represented by 57.0 %. The total abundance of foliage-dwelling predators presented a significant effect of year, being higher in 2020 than in 2021 (P < 0.001). In 2020, both diversification practices influenced the abundance of foliage-dwelling predators. FO plots showed a lower abundance of foliage-dwelling predators, while burclover undersowing significantly decreased their abundance (Tables 1, 2).

In total, we collected 3113 ground-dwelling predators through pitfall trapping in 2020 and 2021. Spiders represented 58.3 % of all individuals followed by rove beetles with 32.3 % and ground beetles with 5.3 %. We identified 15 families of spiders and the family richness of spiders per trap per sampling was on average 0.48 ± 0.06 in 2020 and 0.99 ± 0.06 in 2021. Moreover, we identified a total of 16 ground beetle genera. The genus richness of ground beetles per trap per sampling was 0.2 ± 0.07 in 2020 and 0.7 ± 0.09 in 2021. The statistical analysis of the total abundance of ground-dwelling predators showed a strong interannual variation ($P < 0.001$). Wheat and burclover treatments had no significant effect (Table 1,3).

Table 2 Abundance of foliage-dwelling predator groups (mean individuals per m^2 (± SE) in five wheat treatments (Florence-Aurora, Montcada and Forment monoculture and Florence-Auora with Montcada mixture and Florence-Aurora with Forment mixture) cultivated with the presence (M +) or absence (M -) of burclover undersowing. Bold font indicates significant relationships in the total amount of foliage-dwelling predators according to Tukey-adjusted pairwise comparisons (P < 0.05). Every year was analyzed independently.

Taxonomic group	Burclover undersowing	Florence-Aurora	Montcada	Forment	Florence-Aurora + Montcada	Florence-Aurora + Forment
2020						
Araneae	M +	0.04 ± 0.06	0.1 ± 0.07	0.03 ± 0.02	0.04 ± 0.01	0.1 ± 0.03
	M -	0.1 ± 0.02	0.3 ± 0.03	0.09 ± 0.01	0.1 ± 0.02	0.1 ± 0.02
Coleoptera						
Coccinellidae	M +	0.5 ± 0.01	0.4 ± 0.04	0.1 ± 0.0	0.6 ± 0.01	0.3 ± 0.06
	M -	0.9 ± 0.2	0.5 ± 0.1	0.2 ± 0.03	0.5 ± 0.01	0.3 ± 0.06
Cantharidae	M +	0.1 ± 0.0	0.2 ± 0.05	0.07 ± 0.01	0.1 ± 0.05	0.08 ± 0.03
	M -	0.08 ± 0.08	0.1 ± 0.07	0.03 ± 0.02	0.1 ± 0.04	0.07 ± 0.01
TOTAL		1.7 ± 0,7	1.6 ± 0.4	**0.5 ± 0.2**	1.4 ± 0.5	0.95 ± 0.5
2021						
Araneae	M -	0.07 ± 0.01	0.1 ± 0.03	0.1 ± 0.02	0.1 ± 0.01	0.07 ± 0.01
Coleoptera						
Coccinellidae	M -	0.2 ± 0.04	0.4 ± 0.1	0.2 ± 0.05	0.3 ± 0.09	0.2 ± 0.06
Cantharidae	M -	0.05 ± 0.01	0.09 ± 0.02	0.05 ± 0.01	0.09 ± 0.01	0.05 ± 0.01
TOTAL		0.32 ± 0.02	0.59 ± 0.03	0.35 ± 0.03	0.49 ± 0.05	0.32 ± 0.02

Table 3 Abundance of ground-dwelling predator groups (mean individuals per trap (± SE) in five wheat treatments (Florence-Aurora, Montcada and Forment monoculture and Florence-Auora with Montcada mixture and Florence-Aurora with Forment mixture) cultivated with the presence (M +) or absence (M -) of burclover undersowing.

Taxonomic group	Burclover undersowing	Florence-Aurora	Montcada	Forment	Florence-Aurora + Montcada	Florence-Aurora + Forment
2020						
Araneae	M +	1.1 ± 0.2	1.1 ± 0.3	1.3 ± 0.3	1.0 ± 0.2	1.5 ± 0.3
	M -	0.7 ± 0.2	0.9 ± 0.1	0.8 ± 0.2	1.6 ± 0.4	1.0 ± 0.1
Coleoptera						
Staphylinidae	M +	0.6 ± 0.2	0.5 ± 0.1	0.8 ± 0.3	0.5 ± 0.2	0.8 ± 0.2
	M -	0.7 ± 0.2	0.9 ± 0.3	0.6 ± 0.2	0.4 ± 0.2	0.7 ± 0.4
Carabidae	M +	0.2 ± 0.1	0.02 ± 0.0	0.04 ± 0.0	0.1 ± 0.1	0.2 ± 0.1
	M -	0.1 ± 0.1	0.1 ± 0.04	0.02 ± 0.02	0.2 ± 0.1	0.02 ± 0.0
TOTAL		1.8 ± 0.3	1.8 ± 0.3	1.9 ± 0.5	2.0 ± 0.4	2.2 ± 0.4
2021						
Araneae	M -	2.5 ± 0.5	2.6 ± 0.5	2.8 ± 0.4	2.1 ± 0.3	2.4 ± 0.4
Coleoptera						
Staphylinidae	M -	1.3 ± 0.2	1.2 ± 0.2	2.0 ± 0.6	1.4 ± 0.3	1.4 ± 0.2
Carabidae	M -	0.4 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.2 ±0.1	0.3 ± 0.1
TOTAL		4.2 ± 0.5	4.2 ± 0.6	5.1 ± 0.8	3.8 ± 0.3	4.1 ± 0.4

3.5. Crop yield

Wheat yield was statistically similar in both harvest seasons, and it was not affected by wheat or burclover treatments. However, W×Y interaction was statistically significant (Table 1). This effect was notably observed in FA treatment. In 2021, FA yield decreased by 43.5 % compared to 2020. Moreover, in 2021, FA plots had lower yield compared to other wheat treatments ($P < 0.001$) (Table 4).

Table 4 Crop yield (mean grain kg ha^{-1} ± SE) in five wheat treatments (Florence-Aurora, Montcada and Forment monoculture and Florence-Aurora with Montcada mixture and Florence-Aurora with Forment mixture) cultivated with the presence (M +) or absence (M -) of burclover undersowing. Bold fonts indicate significant differences within wheat treatment according to Tukey-adjusted pairwise comparisons ($P < 0.05$). Every year was analyzed independently.

Burclover Undersowing	Florence-Aurora	Montcada	Forment	Florence-Aurora + Montcada	Florence-Aurora + Forment
			2020		
M-	4380 ± 626	4535 ± 993	3792 ± 219	5765 ± 973	4342 ± 685
M+	5813 ± 631	4084 ± 268	3798 ± 271	3654 ± 855	4453 ± 624
			2021		
M-	**2881 ± 594**	5000 ± 513	5530 ± 572	4459 ± 1036	4106 ± 618

4. Discussion

4.1. The influence of wheat cultivar mixture on aphid abundance and natural enemies.

Our research evaluates the effect of wheat polycultures, specifically two contrasted cultivar mixtures and the incorporation of burclover undersowing, as well as their stacking on aphid control under farming conditions.

The three wheat cultivars used in this study differed in aphid susceptibility. The evaluation of aphid abundance on monoculture plots revealed that Florence-Aurora was more prone to aphid infestation than Montcada and Forment cultivars. As demonstrated in earlier research, one possible reason why Florence-Aurora supports larger aphid populations could be its high tissue nitrogen content (Aqueel & Leather 2011; Nowak & Komor 2010).

Concerning genotypic diversity, we assessed two contrasted mixtures. The first mixture, FAMO, had similar aphid-related functional traits such as odor profile, plant height and nitrogen content (Serra & Àlvaro 2017; Tous-Fandos et al. 2023). This mixture failed to reduce aphid abundance as well as the number of aphids per tiller and, hence, did not offer associational resistance. The second mixture, FAFO combined cultivars with different functional traits, Florence-Aurora and Forment. FAFO plots presented promising aphid control potential in both sampling years because it presented a decrease in aphid abundance. This is the first time that a two-line wheat cultivar mixture provides pest control compared to previous field studies (Mansion-Vaquié et al. 2019).

Additionally, we found a a decrease in the number of aphids per tiller on Florence-Aurora plants grown in FAFO, suggesting lower aphid infestation in Florence-Aurora plants due to bottom-up effects (Barbosa et al. 2009). While acknowledging the potential impact of induced changes in the physiology of the more favored plants, as seen in other studies (Barbosa et al., 2009; Dahlin et al. 2018), it is probable that the associational resistance in FAFO is mediated through odor or physical masking as suggested in the disruptive hypothesis ((Tahvanainen & Root 1972). Mixing Florence-Aurora plants with the less attractive and taller cultivar Forment decreases the likelihood of aphids locating the preferred host, Florence-Aurora. In this regard, previous research has shown that the odor profile of FAFO is less attractive to *Sitobion avenae* compared to the FA odor profile (Tous-Fandos et al. 2023).

Our results agree with previous research wherein the benefits of genotypic diversity on aphid control were cultivar-specific and depended on the identity and interactions between the cultivar mixtures. These findings highlight the importance of selecting favorable cultivar with complementary traits for ensuring functional polycultures (Dahlin et al. 2018; Ninkovic et al. 2002). For the evaluation of top-down effects, we analyzed the parasitism rate and the abundance of beneficial arthropods. Cultivar mixtures did not enhance parasitism rate. Moreover, wheat plots with a higher parasitism rate did not present a lower aphid population. Thus, it is uncertain whether parasitism played a substantial role in aphid control (Helms et al., 2004; Mansion-Vaquié et al., 2019). On the other hand, cultivar mixtures did not have any effect on aphid predators' abundance or richness. Nonetheless, early studies did not find a connection between higher predator abundance and predation rate (Grettenberger & Tooker 2017). Therefore, further research into predation activity is needed to complement the results obtained in this study.

4.2. Burclover undersowing effect on aphid abundance and natural enemies.

A higher ground cover of burclover undersowing was negatively associated with aphid abundance, particularly in FA and FO plots. Cover crops can provide aphid control via bottom-up effects, wherein the chemical and physical concealment of the primary crop diminishes the probability of aphids locating their preferred host plant (Dassou & Tixier, 2016; Hatt et al., 2018; Lopes et al. 2016; Médiène et al. 2011). In this respect, Mansion-Vaquié et al. (2020) demonstrated in a lab experiment that clover undersowing physically obstructed aphid movement, impeding its spread and establishment in wheat crops. In contrast, burclover undersowing slightly affected aphid abundance or the number of aphids per tiller in MO plots. These results suggest that the success of undersowing in enhancing aphid control depends on the characteristics of wheat used and how these plants interact with each other and with aphids.

Furthermore, cover crops may provide shelter and secondary food resources which enhances the abundance of beneficial arthropods (Dassou & Tixier 2016; Gurr et al. 2017). In our research, however, burclover undersowing had no influence on either the total abundance, the richness of aphid predators or the parasitism rate. Possibly because the burclover undersowing did not present attractive floral resources or complex aerial plant architecture (Hatt et al. 2018; Lopes et al. 2016).

4.3. The potential of the stacking genotype and interspecific diversity for aphid control

Some studies have suggested that stacking different levels of crop diversity may lead to higher aphid control by complementary strategies (Hatt & Döring 2023). Nonetheless, the plots with the highest diversity (wheat cultivar mixtures with burclover undersowing) did not outperform the plots with only one diversity practice. Specifically, in our study we observed an unfavorable association between FAFO and burclover undersowing, perhaps because both diversification practices benefited the cropping system by bottom-up effects, which may lead to functional redundancy. These results tied up with previous field research (Mansion-Vaquié et al. 2019). In this regard, many studies have already highlighted the need for complementary ecological processes to ensure functional polycultures (Barot et al. 2017; Gaba et al. 2015).

4.4. Performance of wheat polycultures on yield

Our research addresses the provision of pest control services through diversification practices in real farming conditions, so far overlooked in the scientific literature to date. For that reason, we mixed wheat varieties whose flour is already blended for bread-making production in this region and analyzed the possible effect of diversity strategies on the crop yield. In the 2020 season, yield was comparable among treatments; however, in the 2021 season, cultivar mixtures outperformed modern cultivar Florence-Aurora monoculture plot. We should underscore that in 2021 the region experienced a particularly dry cropping season (c. 80 % reduction in rainfall compared to the mean annual rainfall). Our results indicate the advantages of traditional cultivars and cultivar mixtures over modern cultivar monocultures in maintaining high yields across seasons, regardless of meteorological conditions (Reiss & Drinkwater 2018). Our findings agree with Borg et al. (2018) meta-analyze which showed a grain yield increase of 4.3% to 5.7% in winter wheat mixtures.

When testing the feasibility of a potential weed as a cover crop, it is essential to evaluate its impact on the crop yield to reduce the negative effects of competition. Burclover undersowing, reduced aphid abundance without affecting wheat yield, suggesting a facilitation relationship between the two species (Lopes et al. 2016; Verret et al. 2017). Nevertheless, there are some limitations to the use of burclover in Mediterranean fields, as its establishment and growth are affected by meteorological conditions rendering it unsuitable during dry cropping seasons.

To conclude, this study revealed the potential of genotypic (cultivar mixtures) and interspecific (burclover undersowing) diversity for enhancing bottom-up ecological processes related to aphid population control in organic winter wheat crops. Moreover, it provides significant support for cultivar-specific effects on associational resistance. Cultivar mixture with complementary traits has a great potential on aphid control, while mixing cultivars with similar traits or stacking diversity practices with comparable bottom-up effects do not cause any further reductions in aphid abundance. The advantages of burclover undersowing were found to be closely tied to the identity of the cultivar associated. Thus, trait-approach research is needed to reinforce functional polycultures. Finally, the diverse strategies employed in this experiment had no detrimental effect on crop yields, demonstrating their suitability for polyculture in Mediterranean organic farming conditions.

Acknowledgments

We are extremely grateful to the Gallecs Consortium for its support and the local farmers, especially Salvi Safont for their cooperation throughout the study in agricultural tasks, and to Marc Domènech for identifying the families of ground-dwelling spiders.

Appendix S1

Table S1 Total abundance of the different taxonomic groups (N) collected.

Taxonomic group	Order	Family	Species/Genus	N
Aphids	Hemiptera	Aphididae	*Metopolophium dirhodum*	3920
			Schizaphis graminum	1448
			Sitobion avenae	21357
			Rhopalosiphum padi	632
			Metopolophium festucae	71
			Sipha elegans	58
			unidentified specie 1	1
Parasitoids wasps	Hymenoptera	Braconidae	*Aphidius* sp.	207
			Lysiphlebus sp.	3
			Praon sp.	69
		Encyrtidae	*Syrphophagus* sp.	1
		Figitidae	*Alloxysta* sp.	3
		Megaspilidae	*Dendrocerus* sp.	12
		Pteromalidae	*Asaphes* sp.	36
			Pachyneuron sp.	16
Ground beetles	Coleoptera	Carabidae	*Amara* sp.	2
			Anchomenus sp.	31
			Brachinus sp.	3
			Calathus sp.	5
			Calosoma sp.	3
			Harpalus sp.	18
			Microlestes sp.	21
			Poecilus sp.	2
			Scybalicus sp.	1
			Trechus sp.	57
			unidentified genus 1	16
			unidentified genus 2	2
			unidentified genus 3	2
			unidentified genus 4	1
			unidentified genus 5	1
			unidentified genus 6	1
Ground-dwelling spiders	Araneae	Agelenidae	-	11
		Clubionidae	-	1
		Dysderidae	-	6
		Gnaphosidae	-	99
		Linyphiidae	-	912
		Liocranidae	-	3
		Lycosidae	-	155
		Pholcidae	-	1
		Phrurolithidae	-	9
		Salticidae	-	4
		Tetragnathidae	-	22
		Theridiidae	-	16
		Thomisidae	-	30
		Titanoecidae	-	5
		Zodariidae	-	6

Capítol 3

Influence of plant identity in wheat mixtures on the control of English Grain Aphid (*Sitobion avenae*)

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Influence of plant identity in wheat mixtures on the control of English Grain Aphid (*Sitobion avenae*)

Resum

Els experiments en condicions naturals han demostrat que les barreges de varietats de blat difereixen en la capacitat de regular la població de pugons. Per això, el següent estudi té per objectius avaluar la resistència associativa de dues barreges de blat, així com caracteritzar el contingut de nitrogen tissular i la biomassa aèria, ja que són dos trets funcionals relacionats amb la qualitat de l'hoste i el creixement de la població de pugons.
En experiments d'hivernacle i laboratori s'ha avaluat la taxa d'acceptació de l'hoste, el creixement de la població de pugons i la densitat de pugons de tres varietats de blat cultivades en monocultius o en barreges (Florence-Aurora amb Forment; Florence-Aurora amb Montcada). També s'ha analitzat la biomassa aèria a partir del pes sec i s'ha obtingut el nitrogen tissular mitjançant la combustió de Dumas.
Els resultats demostren que l'eficàcia de les barreges de blat sobre el control de la població de pugons depèn de la identitat de les varietats combinades. L'associació de Florence-Aurora amb Forment va disminuir significativament el creixement de la població de pugons i la densitat de pugons per planta en comparació amb els monocultius, mentre que l'associació de Florence-Aurora amb Montcada no va afectar el desenvolupament de la població de pugons.

En relació amb l'anàlisi de trets funcionals, Forment va presentar menor contingut de nitrogen i biomassa en comparació a Florence-Aurora i Montcada. A més, la interacció planta-planta va modificar els trets funcionals analitzats. El contingut de nitrogen de Florence-Aurora va disminuir en aquelles plantes crescudes conjuntament amb Forment. Aquest fet explicaria la menor densitat de pugons en les plantes de Florence-Aurora crescudes associades amb Forment. D'altra banda, la biomassa de les plantes crescudes en la barreja Florence-Aurora amb Montcada va augmentar respecte les plantes crescudes en monocultiu, fet que podria explicar la major població de pugons observada en la barreja.

En conclusió, els beneficis de les associacions de blat sobre el control dels pugons depenen de la identitat de les plantes combinades. L'estudi sembla indicar que les associacions de varietats amb trets funcionals diferents, com ara Florence-Aurora i Forment, serien més eficients en el control de les poblacions de pugons. Tot i així cal continuar la investigació sobre els atributs relacionats amb la interacció planta-pugó, ja que no es va observar relació entre el contingut de nitrogen i l'abundància de pugons.

1. Introduction

Wheat diversity (i.e. cultivar mixtures) has become an opportunity to introduce plant complexity, improving functionality to organic agricultural systems. This approach also ensures easy management for farmers (Costanzo & Bàrberi 2014; Wezel et al. 2014). The potential of cultivar mixtures to enhance system functionality relies on the presence of extensive genetic variability among cultivars. This diversity imparts to each cultivar a unique and distinctive set of functional traits (Barot et al. 2017). This trait diversity may lead to complementary relationship between cultivars that enhances the provision of functionality and, consequently, agroecosystem services (Gaba et al. 2015; Tilman et al. 2014). For instance, cereal cultivar mixtures have been demonstrated to deliver pest management functions among others benefits (Costanzo & Bàrberi 2014; Tooker & Frank 2012).

In pest management research, aphids are commonly used as model organisms for plant-herbivore studies due to their direct and indirect economic impact on cereal crops. Additionally, their taxonomy, physiology, and life cycle are well understood (Dedryver et al. 2010; Dixon 1987; Rodriguez-Saona et al. 2009). Aphid population control in mixtures essentially relies on associational resistance, which states that beneficial plant-plant interaction between the combined cultivars may lead to a negative impairment of aphid performance (Barbosa et al. 2009; Tahvanainen & Root 1972) The associational resistance can incise in different phases of the aphid host-selection and population development process, as host can be accepted or rejected at any moment of the sequence (Barbosa et al. 2009; Powell et al. 2006). The initial stage of the aphid host-selection and population development process is aphid-host location and detection. During this stage, aphids use olfactory and visual cues to locate their host (Powell et al. 2006). After landing (second phase), aphids use olfactory, gustative, and tactile cues to decide whether or not to accept the plant. The disruptive hypothesis (Tahvanainen & Root 1972) proposes that genotype diversity may enhance associational resistances in the first and second phases, by reducing the likelihood of aphids locating and accepting host-preferred cultivars due to olfactory or visual masking. The third phase corresponds to the aphid colony development. Population growth and aphid size will depend on the quality of the host (Aqueel & Leather 2011; Nowak & Komor 2010). In the third phase, associational resistance may arise because plant-plant interaction via volatiles can cause physiological or morphological shifts in the susceptible cultivar that may ultimately affect the herbivores (Kheam et al. 2023; Ninkovic et al. 2006). For instance, these modifications may alter the host plant quality, or induce herbivore defense mechanisms (Barbosa et al. 2009; Callaway & Walker 1997; Ninkovic et al. 2016).

In this respect, it is necessary to investigate the plant-plant interactions, which can be positive (facilitation, complementation), neutral or negative (competition) in different phases of the aphid host-selection and population development process (Barbosa et al. 2009).

Moreover, it is interesting to assess the role of functional traits on the associational resistance of wheat mixtures (Barot et al. 2017). Earlier research has highlighted the significance of plant odor signals in aphid host identification, location, and acceptance (Pickett et al. 1992; Webster 2012). However, once aphids are established on a plant, their development, survival, and reproduction are mainly driven by plant biomass and quality traits such as tissue nitrogen content (Jakobs & Müller 2018; Szpeiner et al. 2009). Higher plant biomass may have greater herbivore abundance (Barbosa et al. 2009). Furthermore, above-ground plant nitrogen content is a limiting factor for the aphids' diet (Taiz et al. 2007). In this sense, previous greenhouses studies have indicated the positive relationship between nitrogen fertilization and cereal aphid growth rate, individual size and fecundity and size (Aqueel et al. 2014; 2011; Duffield et al. 1997; Gash 2012; Nowak & Komor 2010). Nevertheless, the study of the heterogeneity of tissue nitrogen content across cereal cultivars under identical fertilized conditions and its effect on aphid control has been widely overlooked (Aqueel & Leather 2011; Gaba et al. 2015).

For this aim, we studied the plant-plant interaction on aphid control in different aphid phases, including aphid acceptance and aphid population development of three wheat monocultures Florence-Aurora (*Triticum aestivum* L. subsp. *aestivum*), Forment (*Triticum turgidum* L. subsp. *durum* Desf. (Husn.)) and Montcada (*Triticum aestivum* L. subsp. *aestivum*) and two mixtures: Florence-Aurora with Forment and Florence-Aurora with Montcada. For simplicity, we will refer to Forment as a cultivar thereafter. Although they are taxonomically different species, Triticum aestivum and Triticum turgidum are closely related (Wang et al. 2013). (Wang et al. 2013).

Additionally, we investigate the influence of intraspecific plant interaction on wheat above-ground biomass and tissue nitrogen content. We hypothesize that (i) aphid acceptance will be negatively affected by volatile interactions between cultivars with different odor profile (ii) the capacity of cultivar mixture to impair aphid populations will depend on the identity of the cultivars combined (iii) plant-plant interaction in mixtures will modify wheat above-ground biomass and nitrogen content.

2. Methodology

2.1. Plants and insect material

We set up a greenhouse experiment with three winter wheat cultivars: The modern cultivar Florence Aurora and the traditional cultivars Forment and Montcada. Seeds were supplied by farmers from the Gallecs Agroecological Union (Mollet del Vallès, Barcelona, Spain).

The selection of the cultivars was based on agronomic and commercial criteria to assure the viability of the mixtures in a real farming context.

The wheat cultivars were either grown as monocultures: Florence-Aurora (FA), Forment (FO) and Montcada (MO), or in two cultivar mixtures 1:1 Florence-Aurora and Forment (FAFO) and Florence-Aurora and Montcada (FAMO), summarizing five wheat treatments.

The combination of a modern cultivar with a traditional one was intentional, as modern cultivars are more productive, but more prone to diseases and herbivore attacks, while traditional cultivars can complement the modern cultivar by offering resistance to the agricultural system (Serra-Gironella 2020). Furthermore, we adopted a functional trait approach when selecting cultivars and mixtures. Florence-Aurora and Montcada share similar functional traits related to aphid susceptibility including phenology, height, and odor profile while Forment differs from the others (Serra-Gironella & Álvaro 2017; Tous-Fandos et al., 2023). Hence, we established two contrasted cultivar mixtures, on the one hand, mixing cultivars with homogeneous traits and on the other, combining cultivars with heterogeneous functional traits.

Experimental conditions were established at 18–22 ºC with a light regime of L16: D8 h. Four wheat plants were grown together in a plastic pot (13.7 × 13.7 × 23 cm) in potting soil (Hasselfors P soil, Sweden).

The English grain aphid, *Sitobion avenae* (Fabricius), was reared on oat (*Avena sativa* L. cv. Belinda) in multiclonal cultures in a separate rearing chamber under the same conditions.

2.2. Plant volatile exposure

To analyze aphid acceptance, we exposed tested cultivars (known as called receivers) were exposed to volatiles from inducer cultivars or from the same tested cultivar (self-exposure) as a control. The exposure was carried out in 'two chamber cages' (Ninkovic et al. 2002): Air was drawn into one of the chambers with the inducer cultivar and passed through a hole in the wall into the chamber with the receiver cultivar. The air from the top of the receiving chamber was then drawn out to the vacuum tank and vented outside the greenhouse. The exposure system had an airflow of 1.3 L min 1. Six wheat plants of the same cultivar were planted together in plastic pots (9 × 9 × 7 cm) filled with potting soil (Hasselfors P soil, Sweden). To prevent plant interactions caused by root exudates, the pots were placed in separate Petri dishes. The plants were watered using an automated drop system (DGT Volmatic). Plants at the one-leaf stage were placed in the exposure system and exposed for five days. The experimental plants were grown in a separate growing chamber under the same light and temperature conditions as in the chamber with plant exposure cages, which had an L16: D8 h cycle at 18–22 ºC. To prevent any volatile interactions between cultivars during the pre-exposure period, pots with different cultivars were placed at least 1 meter apart. For aphid settling tests, five of six plants per pot from receiving chambers were randomly chosen. Each combination was represented by four replicates.

2.3. Test of aphid plant acceptance

After plant volatile exposure from the emitting cultivar, plants of the receiving cultivar were tested for aphid acceptance using a no-choice test (Ninkovic et al. 2009). Ten apterous aphids of mixed ages were released into a polystyrene tube (122 × 30 mm) containing the second leaf of a wheat plant. The upper end of the tube was covered by a fine-meshed net and the lower end was plugged with a piece of plastic foam with a slit for the leaf. A wooden stick was used to support the tube. After two hours, the aphids that settled (not walking and with placed antenna over the body) on the tested leaf were counted.

Aphid acceptance may vary between experiments due to small differences in test conditions. To correct for this, At was divided with A0, where At represents the mean number of aphids accepting the volatile-treated plants and A0 represents the mean number of aphids accepting unexposed plants in a test.

Thus, a ratio less than one indicates reduced acceptance, while a ratio of one suggests no induction relevant to aphids occurred, and a ratio greater than one implies an increase in aphid settling.

2.4. Aphid population growth parameters

To assess the growth of aphid population, we conducted an experiment in a greenhouse using 150 pots, with 30 replicates of each wheat treatment. Four weeks after sowing, we inoculated each plant with one aphid and recorded the total number of aphids per plant at 10, 15 and 20 days after aphid inoculation. Aphid population growth was determined using the mean number of aphids per treatment and per sampling time. Twenty days after inoculation, we compared the aphid load, which is defined as the average number of aphids per wheat dry weight, between plants grown in monoculture and those grown in cultivar mixtures. This allowed us to investigate the potential effects of the interaction between cultivars on aphids.

2.5. Wheat biomass analysis

Four weeks after sowing (inoculation time) wheat aboveground biomass was assessed. Additionally, biomass was collected 10- and 20-days post-inoculation from ten pots for each treatment and dried at 60 ºC for 72 hours. In mixture treatments, the two cultivars were weighed separately.

2.6. Wheat nitrogen and carbon content analysis

Before aphid inoculation, we collected and dried the aboveground biomass of four plants from each ten pots in each wheat treatment at 60ºC for 72 h for nitrogen content analysis. The dried samples were ground using an agate-mortar grinder mill. Homogeneous subsamples (2.5–3 µg) of ground wheat tissue were collected for analyses of total N and C content using Dumas’s dry combustion method at the Scientific and Technological Centres of the University of Barcelona (CCiTTUB).

2.7. Statistical analysis

All statistical analyses were conducted using R, version 4.1.1 (R Development Core Team 2021). Differences in aphid acceptance between cultivars exposed to clean air and cultivars exposed to VOCs from other or the same cultivars were analyzed with generalized linear mixed models (GLM) with binomial error distribution in *lme4* (Bates et al. 2015). The results were expressed as the proportion of settled aphids out of 10 introduced aphids as a replicate. The Tukey test was used for post-hoc comparisons.

The measured variables of aphid population growth, aphid load, wheat biomass and wheat nitrogen content were analysed with linear mixed effects models (LMM) or generalized linear mixed effects models (GLMM) using the *glmmTMB* function from the glmmTMB package (Brooks et al. 2017). The best model for each response variable was considered by the Akaike information criterion (AICc). The significance of fixed effect factors and their interactions was determined with an F test with Kenward-Roger approximation for LMMs or a likelihood ratio test (LRT) for GLMMs. Pairwise comparisons were carried out using Tukey-adjusted estimated marginal means (EMMs; a.k.a. least-squares means) from the *emmeans* package (Lenth et al. 2017). Aphid population growth, aphid load and wheat biomass models were fitted considering two fixed covariables: wheat treatment (categorical with five levels: FA, FO, MO, FAFO and FAMO), sampling time (categorical with three levels: 10, 15 and 20 days) and their interactions. The aphid population growth fitted a negative binomial error distribution without interactions. The number of pot replicas per treatment (categorical with 10 levels) was added as a random factor for fitting GLMM with repeated measurements. The aphid load was analyzed independently for each cultivar. Hence, we categorized the wheat treatment factor into two distinct levels: monoculture (FA, MO, or FO) and mixture (FAMO or FAFO). To equalize the sowing ratios between monocultures and mixtures, the total number of aphids per plant in mixture plots was doubled. Aphid load was fitted to a negative binomial GLMM. Wheat biomass model fitted to a Gaussian LMM with interaction between treatment and sampling time. For the statistical analysis of nitrogen, the dataset was separated by mixture. Hence, we assessed Florence-Aurora monoculture, Forment monoculture, and Florence-Aurora with Forment mixture data on the one hand, and Florence-Aurora monoculture, Montcada monoculture, and Florence-Aurora with Montcada mixture data on the other. Wheat nitrogen model included two fixed covariables: wheat treatment (categorical with two levels, monoculture or mixture) and wheat cultivar (categorical with two levels: Florence-Aurora or Forment; Florence-Aurora or Montcada). The wheat nitrogen content model fitted to a Gaussian LMM with interaction between treatment and cultivar factors. The correlation between aphid abundance and the wheat nitrogen content was analyzed via Pearson correlation method.

3. Results

3.1. Aphid plant acceptance

The analysis of aphid plant acceptance indicates that there was no difference in the settlement of *Sitobion avenae* between self-exposed cultivars. However, the mean number of aphids settled on receiving was significantly reduced on Forment plants after its' exposed to volatiles from Florence-Aurora individuals ($F_{4,\ 89}$ =5.74, $P < 0.01$) there in aphid acceptance on Forment individuals exposed to Florence-Aurora volatiles was reduced by 20 % (Table 1). No other significant effects were found.

Table 1 Aphid cultivar acceptance expressed as the proportion between the number of aphids settled on receiver wheat cultivars (Florence-Aurora, Forment and Montcada) after exposure to volatiles from inducer cultivars and unexposed plants. Asterisks indicate a significant shift in aphid cultivar acceptance according to Tukey's-adjusted pairwise test ($P < 0.05$).

	Inducers		
Receivers	Florence-Aurora	Forment	Montcada
Florence-Aurora	0.98	0.98	0.99
Forment	0.80*	0.89	0.94
Montcada	1.06	1.02	0.99

Table 2 Above-ground dry weight of wheat individual (mean ± SE) at 30, 40 and 50 days after sowing grown in monoculture (FA, FO and MO respectively) or in cultivar mixtures Florence-Aurora with Forment (FA in FAFO or FO in FAFO) and Florence-Aurora with Montcada (FA in FAMO or MO in FAMO). Asterisks indicate significant differences within cultivar mixtures according to Tukey's-adjusted pairwise EMMS comparisons ($P < 0.05$).

	FAFO			
	FA	FA in FAFO	FO in FAFO	FO
30 days dw (g)	0.64 ± 0.02*	0.65 ± 0.02*	0.55 ± 0.01	0.54 ± 0.01
40 days dw (g)	2.34 ± 0.06	2.18 ± 0.10	2.11 ± 0.06	2.18 ± 0.04
50 days dw (g)	4.92 ± 0.05	4.76 ± 0.11	5.07 ± 0.28	5.24 ± 0.16*
	FAMO			
	FA	FA in FAMO	MO in FAMO	MO
30 days dw (g)	0.64 ± 0.02*	0.80 ± 0.01	0.73 ± 0.01	0.62 ± 0.02*
40 days dw (g)	2.34 ± 0.06	2.39 ± 0.06	2.72 ± 0.11	2.56 ± 0.04
50 days dw (g)	4.92 ± 0.05*	5.08 ± 0.09	6.08 ± 0.14	5.63 ± 0.29

3.2. Aphid population development

The total number of aphids per pot was used to calculate the size of the aphid populations in monocultures and mixtures. The population size increased over time in all wheat treatments ($\chi_{2, 90} = 2538.91$, $P < 0.001$). Aphid population size differed between wheat treatments ($\chi_{4, 88} = 24.20$, $P < 0.001$). From the first counting, the FA cultivar supported the greatest aphid population, being significantly higher than FAFO ($P < 0.001$) at 20 days after inoculation (Fig. 1).

The aphid load was lower in plants grown in mixtures compared to monocultures for all three tested cultivars. Florence-Aurora and Forment individuals grown in the FAFO mixture had a significantly lower aphid load (FA: $\chi_{2, 60} = 7.14$, $P < 0.05$, FO: χ 1, 72 = 10.83, $P < 0.01$). Similar results were observed in Montcada individuals grown in FAMO ($\chi_{1, 72} = 10.23$, $P < 0.01$). However, the aphid load in Florence-Aurora and Montcada plants grown in FAMO mixture was comparable to those plants grown in monoculture (FA: $\chi_{2, 60} = 3.04$, $P = 0.09$, MO: $\chi_{1, 72} = 5.41$, $P = 0.11$) (Fig. 2).

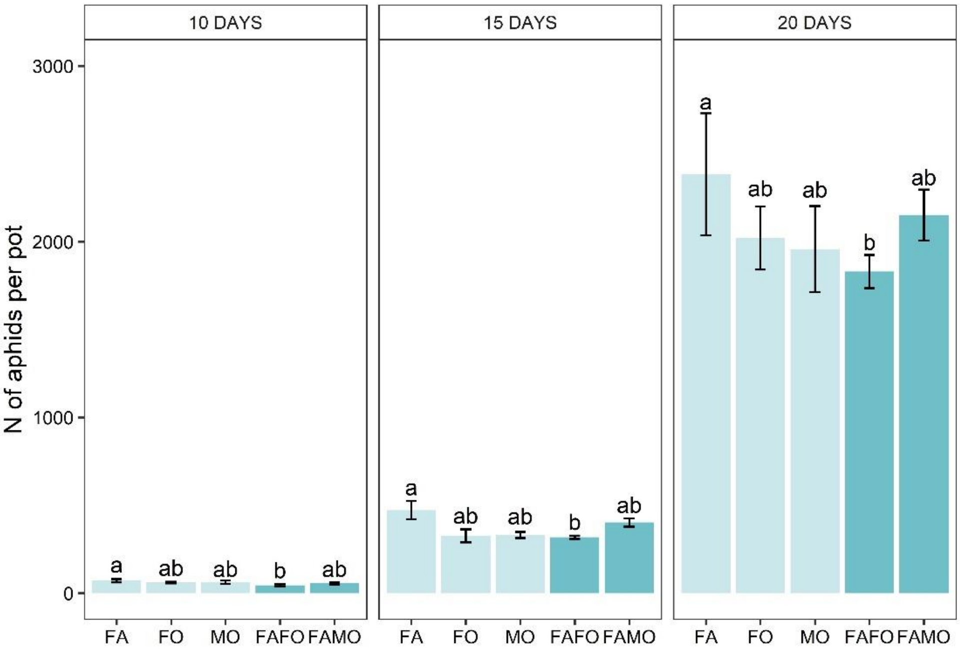

Figure 1 Aphid population growth over time (mean ± SE) at 10, 15 and 20 days after aphid inoculation on five wheat treatments: three monocultures, Florence-Aurora (FA), Forment (FO) and Montcada and two cultivar mixtures, Florence-Aurora with Forment (FAFO) and Florence-Aurora with Montcada (FAMO). Different letters indicate significant differences between wheat cultivars and mixtures within each sampling time according to Tukey-adjusted pairwise EMMS comparisons ($P < 0.05$).

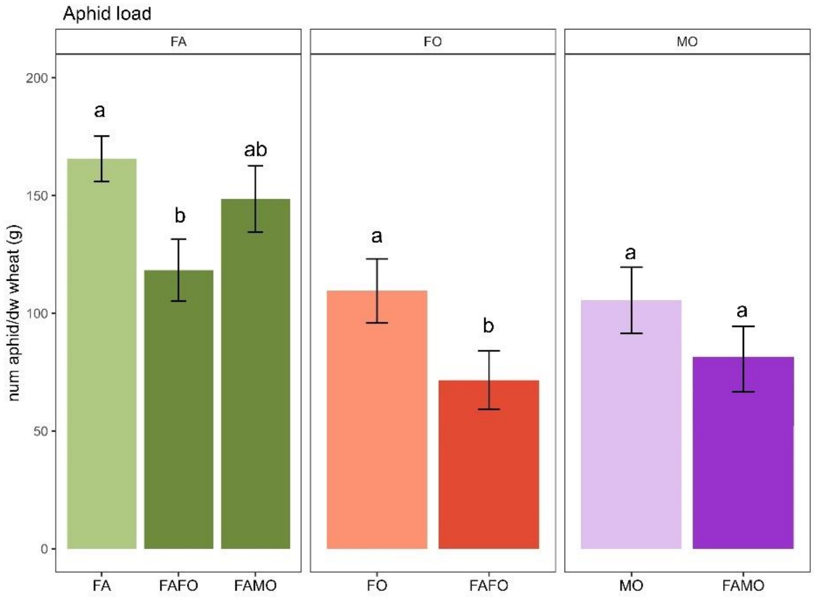

Figure 2 Aphid load (mean number of aphids per wheat dry weight ± SE) at 20 days after inoculation; a) Florence-Aurora cultivar grown in monoculture (FA), mixed with Forment (FAFO) and mixed with Montcada (FAMO); b) Forment cultivar grown in monoculture (FO) or mixed with Florence-Aurora (FAFO); c) Montcada cultivar grown in monoculture (MO) or mixed with Florence-Aurora (FAMO). Different letters indicate significant differences according to Tukey's-adjusted pairwise EMMS comparisons (P< 0.05).

3.1. Wheat biomass

At 30 days and 50 days after sowing, the above-ground biomass of cultivars grown in monocultures was significantly different (30 days: $F_{1,3} = 3.34$, $P < 0.05$, 50 days: $F_{1,3} = 2.21$, $P < 0.05$). Florence-Aurora and Montcada had greater biomass than Forment at 30 days after sowing, ($P < 0.05$) while at 50 days Forment presented higher wheat biomass than Florence-Aurora ($P < 0.05$). The wheat above-ground biomass was affected by plant-plant interaction in cultivar mixtures ($F_{1,3} = 5.48$, $P < 0.05$). At 30 and 50 days after sowing, plants grown in FAMO mixture had greater above-ground biomass when compared to monocultures ($P < 0.05$) (Table 2).

3.2. Nitrogen content

The N content of aboveground tissue in one-month-old wheat plants grown in monoculture varied between cultivars. Florence-Aurora had a higher N content than Forment monocultures ($F_{1,3} = 4.94$, $P < 0.05$). The N content of Florence-Aurora decreased significantly when grown together with Forment ($F_{1,3} = 5.91$, $P < 0.05$) resulting in a similar N content of the two mixed cultivars. In contrast, Florence-Aurora and Montcada cultivars had similar N content when grown in monocultures ($F_{1,3} = 0.14$, $P = 0.71$), and when mixed ($F_{1,3} = 0.24$, $P = 0.62$). Neither Forment nor Montcada tissue N content varied due to growth in the mixture (Table 3). The results showed a low positive correlation between the number of aphids per plant and aboveground nitrogen content (r = 0.30, P = 0.02). The carbon content of aboveground tissue was not impacted by the wheat cultivars, whether grown in monocultures or mixtures (Table 3).

Table 3 Total percentage of nitrogen (N %) and carbon (C %) (mean ± SE) of wheat tissue of Florence-Aurora, Forment and Montcada cultivars grown in monocultures (FA, FO and MO respectively) or in cultivar mixtures Florence-Aurora with Forment (FA in FAFO or FO in FAFO) and Florence-Aurora with Montcada (FA in FAMO or MO in FAMO). Asterisks indicate significant differences within cultivar mixtures according to Tukey's-adjusted pairwise EMMS comparisons (P < 0.05).

		FAFO		
	FA	FA in FAFO	FO in FAFO	FO
N (%)	5.28 ± 0.07*	5.08 ± 0.05	5.06 ± 0.02	5.09 ± 0.05
C (%)	36.31 ± 0.15	36.88 ± 0.12	36.54 ± 0.18	36.70 ± 0.39
		FAMO		
	FA	FA in FAMO	MO in FAMO	MO
N (%)	5.28 ± 0.07	5.16 ± 0.08	5.29 ± 0.08	5.22 ± 0.05
C (%)	36.31 ± 0.15	36.61 ± 0.22	36.70 ± 0.39	36.51 ± 0.20

4. Discussion

4.1. Genotypic diversity benefits on aphid control are mixture specific.

For this study, it was of interest to investigate the performance of two wheat cultivar mixtures on pest management. We conducted the experimentation in a greenhouse because it enables us to control the environmental factors and center the research uniquely on the influence of plant-plant interaction on aphids.

On early phases of aphid host detection, plant communication through volatiles affected aphid responses. Aphid acceptance decreased when the inducer and the receiver plants had distinct odor profiles. This effect was only observed in Forment plants exposed to Florence-Aurora volatiles. It is interesting to note that the effect was not bilateral, hinting at the specificity of plant communication. The reduced acceptance of aphids may be due to a physiological modification of the receiving plants, making them less attractive to aphids (Kheam et al. 2023). Another possible explanation is that the aphid detected the changes of volatiles of exposing plants and interfered with the location and acceptance of the host (Kheam et al. 2024).

Regarding aphid population development, our results revealed that the effectiveness of a mixture to deliver aphid control depended on the identity of the combined plants, supporting the right neighbor concept (Dahlin et al. 2018; Kheam et al. 2023). The Florence-Aurora and Forment mixture reduced aphid populations compared to monocultures and resulted in a significantly lower aphid load on plants. In contrast, the mixture of Florence-Aurora and Montcada cultivars did not affect aphid population development. One possible explanation of the contrasted performance of the two studied mixtures could be that combining plants with different functional traits, such as tissue nitrogen content and above-ground biomass, as seen in the Florence-Aurora with Forment mixture, may enhance facilitation interactions and thus, associational resistance. However, the association of similar cultivars may lead to functional redundancy.

4.2. The effect of quality traits on the control of aphid populations in wheat stands.

There is a considerable body of literature relating plant nutritional parameters, such as plant biomass and tissue nitrogen content with aphid performance (Jakobs & Müller 2018; Duffield et al. 1997; Gash 2012; Dixon 1987). Wheat cultivars with greater biomass can sustain a larger aphid population (Szpeiner et al. 2009), while high nitrogen fertilization inputs are known to boost aphid populations, leading to yield grain loss (Duffield et al. 1997; Gash 2012). Cereal aphids, such as *Sitobion avenae* and *Metopolophium dirhodum* (Walker), have been found to increase their size, longevity and fecundity in high nitrogen fertilized crops, which enhances their performance (Aqueel & Leather 2011; Duffield et al. 1997; Nowak & Komor 2010). Our results indicated that wheat cultivars, particularly Forment, differed in both above-ground biomass and nitrogen content under the same soil nutrition conditions demonstrating the heterogeneity of functional traits related to aphid susceptibility within wheat cultivars. It is important to highlight that the benefits of genotypic diversity on pest management rely on the idea of complementary functions between cultivars (Barot et al. 2017).

Furthermore, our findings supported the idea that intraspecific interactions can lead to plant physiologic modifications. Previous studies have demonstrated that interaction between plants through volatile compounds can cause shift in both physiological and morphological characteristics (Barbosa et al. 2009; Dahlin et al. 2018; Ninkovic et al. 2016). For instance, Tous-Fandos et al. (2023) demonstrated that volatile interaction in wheat cultivar mixtures can alter the odor profile of the mixture. Dahlin et al. (2020) demonstrated that cultivar mixtures can affect the plasticity of SLA (Specific Leaf Area), height, and phenology traits. Our observations revealed that mixing cultivars with similar above-ground biomass, such as Florence-Aurora and Montcada cultivars, increased the average biomass of the plants. This greater above-ground biomass, and hence the possibility to sustain larger aphid populations, may be one of the reasons why Florence-Aurora with Montcada mixture was not effective on aphid control (Szpeiner et al. 2009).

The nitrogen content varies depending on the neighboring plant identity. In this case, Florence-Aurora's tissue nitrogen content decreased significantly when mixed with Forment but remained constant when combined with Montcada. This reduction in tissue nitrogen content in Florence-Aurora plants grown with Forment mixture may result in the diminishing aphid load found in those Florence-Aurora plants.

This study revealed that plant-plant interaction in wheat mixtures results in physiological modifications of functional traits. Our findings suggested that combining cultivars with different traits, as in Florence-Aurora and Forment, may enhance positive interactions that boost the associational resistance of the mixture. (Barbosa et al. 2009). Conversely, mixing cultivars with similar traits may result in neutral or even negative interactions that can minimize the beneficial effects of some wheat cultivar mixtures on pest management, as described in the right neighbor hypothesis.

Finally, the correlation between nitrogen content and aphid abundance was low, suggesting that other factors may also contribute significantly to the development of the aphid population in the mixture. Previous studies have highlighted the importance of specific phloem sap composition for host acceptance and performance of phloem-feeding insects (Nowak & Komor 2010; Karley et al. 2002; Gallinger & Gross 2020; Jakobs et al. 2019). Jakobs and Müller (2018) found that generally high amino acid concentrations, hence nitrogen, do not necessarily benefit aphid performance and development.

5. Conclusion

This study highlights the importance of neighbor's identity and trait approach in assessing the potential of genotypic diversity for aphid control. In this regard, further investigation of the right neighbor's concept, identification of intraspecific interactions in mixtures and the underlying mechanisms leading to associational resistance are crucial. In this sense, future research on the heterogeneity of phloem sap quality, including amino acids, sucrose, and organic acids, among wheat cultivars may prove important in identifying functional traits for pest control.

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Capítol 4

Alterations in the odor profile of plants in cultivar mixtures affect aphid host-location behavior

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Alterations in the odor profile of plants in cultivar mixtures affect aphid host-location behavior

Resum

Les plantes emeten constantment senyals olfactius per comunicar-se. Els pugons usen aquests senyals per identificar i localitzar els seus hostes. Per tant, els senyals olfactius poden ser un tret funcional clau pel control de la població de pugons. Amb la finalitat d'aprofundir en aquesta recerca, el següent estudi caracteritza el perfil olfactiu de varietats de blat quan creixen en monocultiu i associades a altres varietats (Florence-Aurora amb Forment i Florence-Aurora amb Montcada), així com la resposta d'atracció o rebuig dels pugons vers els senyals olfactius emesos. El monocultiu de Forment va presentar un perfil olfactiu diferent del dels monocultius de Florence-Aurora i Montcada. L'associació de varietats amb un perfil olfactiu diferent, Florence-Aurora amb Forment, va modificar la quantitat emesa d'alguns compostos volàtils i va alterar la proporció del perfil olfactiu de la barreja. L'olor de l'associació de Florence-Aurora amb Forment va ser menys atractiva per l'espècie de pugons analitzada (*Sitobion avenae*) en comparació als senyals olfactius dels monocultius i, fins i tot, en comparació de la combinació de les olors dels dos monocultius. Així doncs, aquest estudi demostra que part de la resistència associativa observada en barreges de varietats de blat pot ser generada per modificacions del perfil olfactiu de la barreja que resulta menys atractiva pels pugons. Tot i això, aquest efecte depèn de la identitat de les varietats associades i és més plausible en associacions de varietats amb perfil olfactiu diferenciat.

A l'apèndix es proporciona la versió publicada del article.

1. Introduction

As sessile organisms, plants are constantly exposed to several threats, such as adverse ambient conditions, resource competition, and herbivore attacks; hence, they have developed highly sophisticated strategies to guarantee their survival. For instance, plant-plant interaction is a key mechanism for enhancing plant fitness in competitive scenarios, such as cultivar mixtures (Ninkovic et al. 2016). Such interactions between cultivars can lead to unfavorable (associational susceptibility) or favorable (associational resistance) associations for neighboring plants that influence their susceptibility to herbivorous insects (Barbosa et al. 2009).

The effect of intraspecific plant diversity on aphid control has been attributed to associational resistance. This suggests that combining the right cultivars reduces herbivorous damage to the host-preferred cultivar by inducing competition-related changes that affect herbivore feeding. These may include the production of anti-herbivore defenses, alteration of host plant quality, or interference in the host location of herbivores(Barbosa et al. 2009).

Furthermore, several studies have demonstrated that the benefits of genotypic diversity are cultivar-specific and depend on the interactions between the cultivar mixtures, leading to the "right neighbor" concept (Dahlin et al. 2018; Kheam et al. 2023). For instance, Ninkovic et al. (2002) tested the aphid control effect of barley cultivar mixtures in a field experiment using various pairs of barley cultivars; they demonstrated that only certain combinations decreased aphid acceptance.

Host selection in aphids is an extremely complex process that involves a variety of sensory and behavioral mechanisms (Powell et al. 2006). Previous studies have emphasized the importance of plant odor signals in aphid host identification, location, and acceptance (Pickett et al. 1992; Webster 2012). Volatile organic compounds (VOCs) are detected via the antennal olfactory sensilla and can be used to locate host plants prior to settling to determine the quality of the phloem composition (Powell et al. 2006). Hence, understanding the role of plant olfactory cues in aphid host location behavior may lead to improved integrated pest management strategies.

Furthermore, it is well-documented that plants respond to neighboring volatiles, causing morphological and physiological modifications (Ninkovic et al. 2016). For instance, VOCs from herbivore-damaged plants can trigger defensive responses in neighboring undamaged plants (Midzi et al. 2022). Furthermore, VOCs from undamaged plants can induce physiological shifts in neighboring plants, increasing cultivar resistance to aphids or altering tritrophic interactions (allelobiosis) (Ninkovic et al. 2006; Dahlin et al. 2018; Kheam et al. 2023).

Previous studies have mostly focused on volatile interactions between cultivars and their implications for aphid acceptance (Dahlin et al. 2018; Ninkovic et al. 2002). Less is known about the complete plant-plant interactions when grown together, their physiological response, and their consequences on allelobiosis. Hence, this study sought to investigate these aspects. Additionally, gaining a deeper understanding of the physiological mechanisms underlying associational resistance may serve as a foundation for enhancing “right neighbor” combinations and, by extension, as a design tool for functional agrobiodiversity (Barbosa et al. 2009; Gaba et al. 2015; Borg et al. 2018).

Therefore, this study aimed to assess the influence of overall above- and below-ground wheat cultivar interactions, when grown together to simulate field conditions, on the mixture odor profile and its subsequent effect on aphid host location behavior. Two previously field-tested wheat mixtures with varying aphid control capacities were compared. We hypothesized that (i) genotypic diversity effects on aphid host location would be specific to the cultivars combined, (ii) the coexistence of cultivars in the mixture would induce changes in their volatile profiles, and (iii) aphids would prefer odor cues from monocultures over those from cultivar mixtures.

2. Methodology

2.1. Plants and insect material for experiments

Three winter wheat cultivars were used in the experiments: the modern cultivar Florence-Aurora (*Triticum aestivum* L. subsp. *aestivum*) and the traditional cultivars Forment (*Triticum turgidum* L. subsp. *durum*) and Montcada (*Triticum aestivum* L. subsp. *aestivum*). Seeds were supplied by farmers from the Gallecs Agroecological Union and technical personnel from the Gallecs Natural Interest Area Consortium in (Catalonia, Spain). The three wheat cultivars were either grown as monocultures: Florence-Aurora (FA), Forment (FO), and Montcada (MO), or in two cultivar mixtures: 1:1 Florence-Aurora and Forment (FAFO) and Florence-Aurora and Montcada (FAMO). The selection of cultivars and mixtures was based on the farmers’ preferences, with Florence-Aurora being the principal cultivar owing to its excellent bread-making qualities. Both mixtures have exhibited contrasting aphid control abilities in previous field studies (unpublished data). Florence-Aurora was treated as the principal cultivar because it is the most cultivated cultivar in the Gallecs Agroecological Union. Four wheat plants were planted together in plastic pots (13 × 13 × 23) in potting soil (Hasselfors Garden P soil). The plants were grown under controlled conditions in a growth chamber at 18–21 °C with a 16/8-h light/dark cycle. Olfactometer experiments and headspace collections were conducted on 1-month-old wheat plants. The English grain aphid *Sitobion avenae* (Fabricius) was reared on oats (*Avena sativa* L. cv. Belinda) in multiclonal cultures in separate rearing chambers under identical conditions.

2.2. Aphid olfactory response

Aphid olfactory responses to different wheat odors were examined using a two-way airflow olfactometer, which consisted of two stimulus zones in which the odors were introduced and a central zone separating them. Airflow through the system was set to 180 ml min-1 measured with a flow meter at the arm inlets (Ninkovic et al. 2013). Plants used as odor sources were placed inside chamber cages directly connected to the olfactometer arms. A wingless S. avenae adult was carefully inserted in the middle of the olfactometer using a fine brush. After acclimatization for 10 min, the position of each aphid was recorded at 3 min interval for 30 min. Each aphid was used only once and was regarded as a replicate. Olfactometers were cleaned with 70% ethanol between trials. Aphids that did not move after acclimatization were excluded from the analysis. the experiment was conducted in a dark room under artificial light (Osram FQ80W/840 HO Constant Lumilux Cool White (4000 K); Munich, Germany) at 60 µmol m-2 s-1 above the olfactometer to limit the influence of visual inputs. Further, the experiments were conducted on sunny spring days from 9:00 am to 4:00 pm. The average room temperature was 20 C and relative humidity was 40-50%.

First, we compared the monocultures with each other or their respective cultivar mixtures (FA vs. FO, FA vs. MO, FA vs. FAFO, FO vs. FAFO, FA vs. FAMO, and MO vs. FAMO; Figure 1a). Second, we investigated the olfactory responses of aphids to mixed odors from the monocultures. To mix the odor of plants from two pots, each pot was placed in a separate cage, but both cages were connected by a Y-connector to the same olfactometer inlet, resulting in a mixed odor of the two monocultures (FA + FO and FA + MO). Through this method, the odors of the two separately grown monocultures could be introduced on one side of the olfactometer and tested against clean air. Thus, two plants were used as one odor source. We offered this mixed odor simultaneously with the odors of the corresponding cultivars grown in a mixture (FA + FO vs. FAFO and FA + MO vs. FAMO). To equalize the number of plants (biomass), two pots of the cultivar mixtures were connected to an olfactometer, as described for the monocultures (Figure 1b). For the avoidance test, we compared cultivar mixtures (FAFO and FAMO) and mixed odors from monocultures (FA + FO and FA + MO) with clean air (Figure 1c). Each treatment comparison was replicated with 14–27 aphids. Data were analyzed using Wilcoxon matched-pair tests in R, version 4.1.1 (R Development Core Team 2021).

2.3. VOC collection

To avoid volatile interactions between the plants, each pot with wheat plants was grown separately inside clear Perspex chamber cages (10 × 10 × 80 cm) (Ninkovic et al. 2002). Air was allowed to enter the forward chamber through an opening in the cage wall (7 cm in diameter), extracted through a tube attached to a vacuum tank, and vented outside the room using an electric fan. The airflow through the cages was 1.3 L min -1. Volatiles were collected using a push-pull system. The upper part of the pot containing the four wheat plants was placed inside a polyethylene terephthalate oven plastic bag (Toppits®; Melitta, Minden, Germany). A self-packed glass liner containing 50 mg of the molecular absorbent Tenax TA (GLScience, Veldhoven, Netherlands) was inserted into the upper opening of the bag. Ambient charcoal-filtered air was pushed into the bag through a Teflon tube inserted into a small hole in the bottom at a flow rate of 600 mL min -1 and pulled out over the absorbent at 400 mL min-1. Volatiles were collected for 24 h. At least 10 replications were performed per treatment.

After the collection of VOCs, the aboveground dry weight was measured by cutting all plants per pot above the soil, drying them for 72 h at 60 °C, and weighing them afterwards. Volatile samples were analyzed using gas chromatography/mass spectrometry (GC-MS). The sampling tubes were inserted in an Optic 3 Injector (GLScience, Eindhoven, Netherlands), which was heated from 40 °C up to 250 °C at 30 °C /sec to release the volatiles from the absorbent. Helium was used as carrier gas (Helium 6.0) with a flow of 1.3 mL min 1. The thermal desorbed compounds were separated using an Agilent 7890 N GC system equipped with an HP-1MS capillary column (30 × 0.25 mm inner diameter × 0.25 μm film thickness, 100% dimethylpolysiloxane) and coupled with an Agilent 5975C mass spectrometer (Agilent Technologies, Inc., Santa Clara, CA, USA). The GC temperature program was as follows: Initial oven temperature of 30 °C was held for 2 min, increased at a rate of 5 °C min-1 to 150 °C, followed by an increase at a rate of 10 °C min-1 to the final temperature of 250 °C, and then held for 15 min. The temperature of the MS ion source was maintained at 230 °C. The quadrupole mass detector was operated in electron impact (EI) mode at 70 eV. The MS gain was set to 10. All data were obtained by collecting the full-scan mass spectra within the range of 40–500 m z-1. Authentic standards of volatile compounds for identification were measured under the same GC-MS conditions.

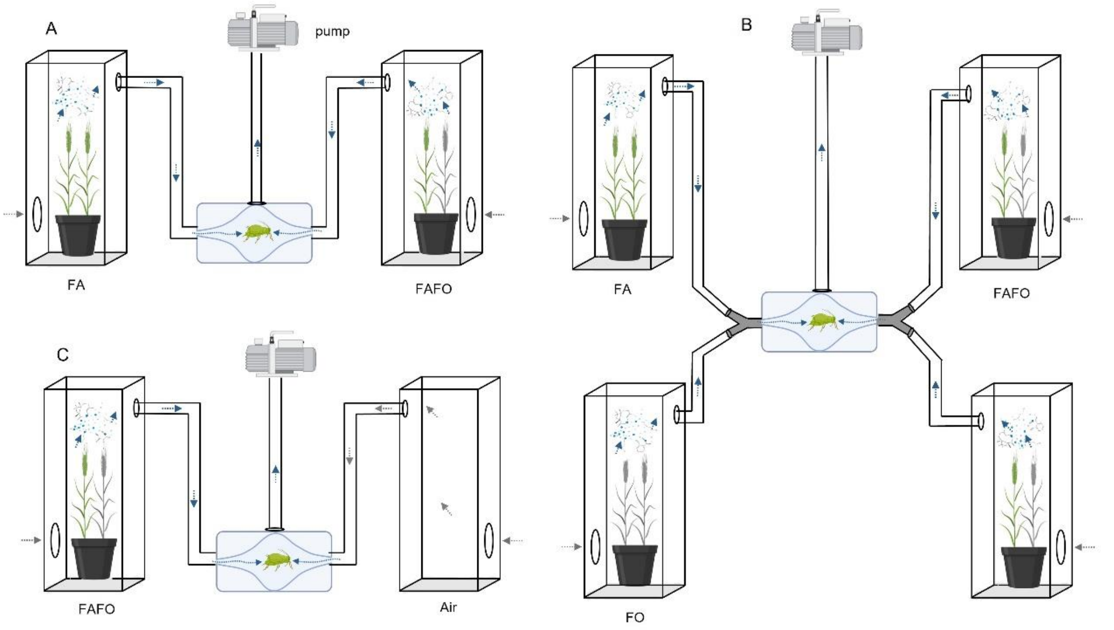

Figure 1 Schematic of the experimental design of the olfactometer assays. Pots with four wheat plants were placed in each cage connected to an olfactometer and a suction pump that was used to facilitate airflow from the plants through the olfactometer. An apterous adult aphid was placed in the middle of the olfactometer. (a) Representative single-pot comparisons between monocultures and their respective mixtures. (b) Representative comparison of two versus two pots. To compare mixed odors of monocultures (one cultivar per pot/cage) and their mixture (two pots/cages of mixtures). Two cages were connected using Y-connectors to mix volatile cues

before entering each olfactometer arm. (c) Schematic of the avoidance test. Aphid movement towards plant odors was compared with clean air. Wheat cultivars: FA = Florence-Aurora, FO = Forment, MO = Montcada.

2.3.1. Identification and Quantification with AMDIS

Volatile compounds from the chromatograms were identified and quantified using the Automated Mass Spectral Deconvolution and Identification System (AMDIS, V. 2.71; National Institute of Standards and Technology, Boulder, CO, USA)., Compound identification was based on the comparison of ion fragmentation patterns and retention indices (RI) of reference standards. Compounds where no standards were available were annotated as unknowns and their ion fragmentation pattern and RI were used to ensure the comparison of the same compounds between samples, according to the protocol by Gross et al. (2019). After deconvolution, peak areas were integrated for quantification. Identification and deconvolution criteria were applied as follows: match factor, ≥75%; relative retention index deviation, ≤5% from reference value, match factor penalties level, very strong; maximum penalty, 20; component width, 12; adjacent peak subtraction, 1; resolution, low; sensitivity, very low; shape requirements, high; . Components with a signal-to-noise ratio of <300 were excluded from the analysis.

2.3.2. Statistical analysis

The overall volatile composition of different wheat monocultures and wheat cultivar mixtures as well as the amounts of individual volatiles were compared using multivariate analysis in R (R Development Core Team, 2021).

To compare the volatile composition, a Bray–Curtis dissimilarity matrix was calculated using the vegdist function from the ‘vegan’ package (Oksanen et al., 2022). Permutational multivariate analysis of variance (PERMANOVA) of the dissimilarity matrix was calculated using the adonis2 function (N permutations = 10.000). This was followed by pairwise comparisons between wheat monocultures and mixtures using pairwis.perm.manova of the ‘RVAideMemoire’ package (Hervé et al., 2022). P-values were adjusted using the Bonferroni method.

The dissimilarities between the VOC profiles of wheat treatments were visualized with a non-metric multidimensional scaling (NMDS) plot generated with the metaMDS function (‘vegan’). We used two dimensions (k = 2) and Wisconsin’s double standardization for scaling.

The amount of single volatiles released by wheat plants was analyzed as the peak area/dry weight (g) of above-ground biomass. Kruskal–Wallis rank sum tests were performed to determine the significant differences in the release of single volatiles between wheat treatments. Pairwise comparisons were performed using Dunn’s test. P-values were adjusted using the Bonferroni method.

3. Results

3.1. Olfactometer test

To identify the effects of the cultivar mixtures on the olfactory response of aphids, olfactometer experiments were conducted with apterous aphids. When the odors of single wheat cultivars grown as monocultures were offered simultaneously, aphids did not show any preference for one of the cultivars (FA vs. FO: Wilcoxon test: Z = 0.67, P = 0.5, n = 17; FA vs. MO: Z = 1.11, P = 0.26, n = 15). Regarding cultivar mixtures, aphids were more attracted to the odor of FA monocultures than to those of FAFO mixtures when offered simultaneously (Z = 2.78, P < 0.01, n = 25) (Figure 2a). No preference was observed for the FO monoculture odors over those from the FAFO mixture (Z = 0.88, P = 0.38, n = 27). (Figure 2b).

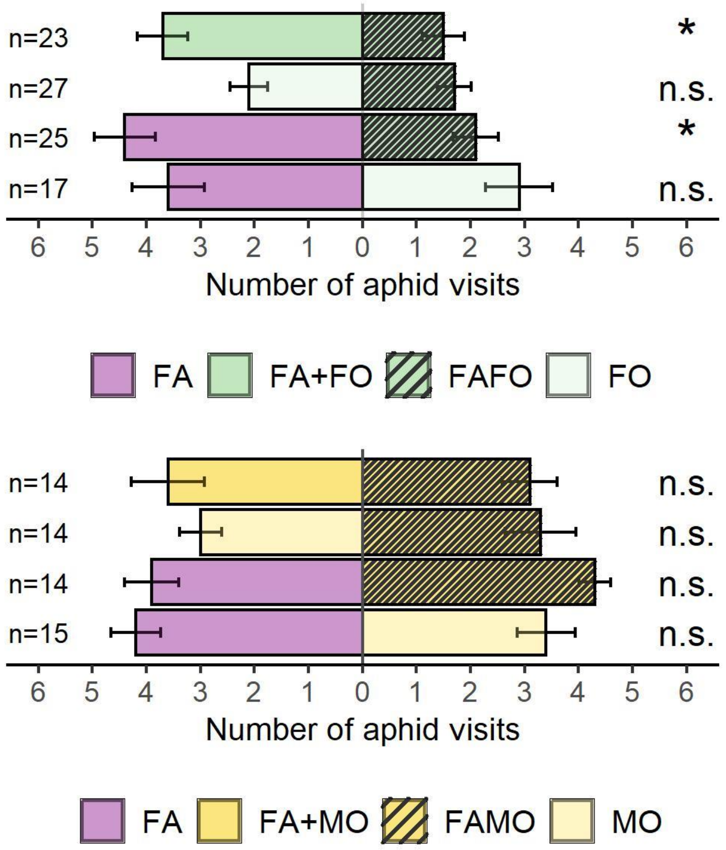

Figure 2 Number of aphid visits to wheat plant odor cues. (A) Comparisons related to FAFO mixture: FA + FO (odor mixture of Florence-Aurora and Forment cultivars when grown in monoculture) vs. FAFO (Florence-Aurora and Forment mixture) (n = 23), FO (Forment monoculture) vs. FAFO (n = 27), FA (Florence-Aurora monoculture) vs. FAFO (n = 25), FA vs. FO (n = 17). (B) Comparisons related to FAMO mixture: FA + MO (odor mixture of Florence-Aurora and Montcada when grown in monoculture) vs. FAMO (Florence-Aurora and Montcada mixture) (n = 14), MO (Montcada monoculture) vs. FAMO (n = 14), FA vs. FAMO (n = 14), FA vs. MO (n =

15). Error bars indicate standard error of the mean. Asterisks indicate significant differences according to the Wilcoxon signed-rank test (P < 0.05).

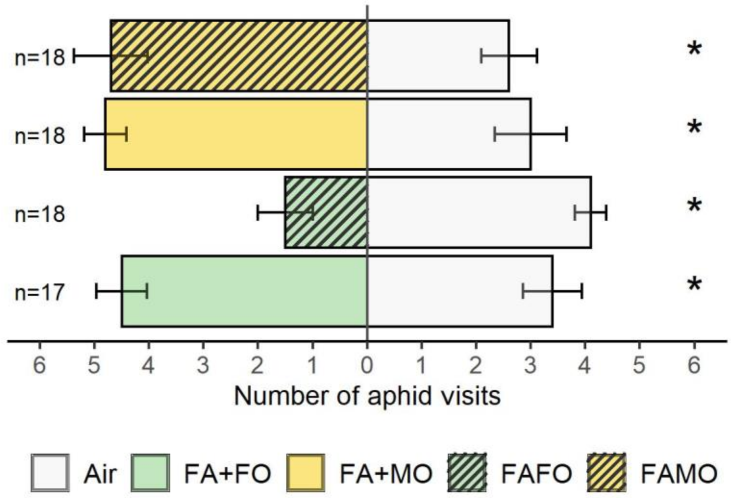

Figure 3 Number of aphid visits to wheat plant odor cues and clean air. Wheat treatments: FA + FO (odor mixture of Florence-Aurora and Forment when grown in monoculture) (n = 17), FA + MO (odor mixture of Florence-Aurora and Montcada when grown in monoculture) (n = 18), FAFO (Florence-Aurora and Forment mixture) (n = 18) and FAMO (Florence-Aurora and Montcada mixture) (n = 18). Error bars indicate standard error of the mean. Asterisks indicate significant differences according to the Wilcoxon signed-rank test (P < 0.05).

To evaluate whether the decrease in attraction for the FAFO mixture odors was due to changes in VOC emission when Florence-Aurora and Forment cultivars grew together or as a mixture of odors from FA and FO, we compared the mixed odors from monocultures (FA+FO and FA+MO) against odors from the cultivar mixture (FAFO and FAMO). Aphids preferred the odor cues from FA + FO over those from FAFO (Z = 2.72, P < 0.01, n = 23) (Figure 2a). In contrast, the FAMO mixture had no effect on aphid behavioral responses (Fig. 2B). In the avoidance test, FAFO was the only treatment that elicited aphid avoidance (Z = 2.63, P < 0.01, n = 20). As expected, in the remaining treatments, aphids were significantly more attracted to plant odor cues than to clean air: FA + FO (FA + FO vs. air: Z = 2.43, P = 0.05, n = 19), FA + MO (FA + MO vs. air: Z = 2.09, P = 0.05, n = 20), and FAMO (FAMO vs. air: Z = 2.31, P = 0.05, n = 20) (Figure 3).

3.2. VOCs profile

To investigate the impact of intraspecific interactions between wheat cultivars on the volatile emission, the headspace of wheat plants of three cultivars grown as monocultures or in a two-way mixture was sampled and analyzed via TD-GC-MS.

We analyzed the composition of 88 detected compounds emitted by wheat plants. The volatile compositions differed significantly between cultivars in monocultures and mixtures (PERMANOVA, df = 4, R2 = 76.13, N = 10.000, P < 0.001). Pairwise comparisons showed significant differences in odor profiles of Florence-Aurora and Forment in monoculture (P < 0.01) as well as compared to the FAFO mixture (Figure 4). Odor compositions of the FAMO mixture were not distinguishable from the odor profiles of FA and MO monocultures (Figure 4).

Overall, cultivar mixtures released higher amounts of VOCs than the three monocultures (Figure 5). Particularly, FAFO emitted significantly higher amounts of 33 specific compounds when compared to FA (37.5 %) and of 40 compounds when compared to FO (45.4 %), including β-caryophyllene, β-ocimene, limonen, 1-octen-3-ol, nonanal, octanal, benzaldehyde, acetophenone among other unknown compounds (Figure 5). VOC emission from FAMO mixture was similar to FA and MO monocultures, only releasing a significantly higher amount of 8 (9.09 %) and 2 (2.27 %) compounds, respectively.

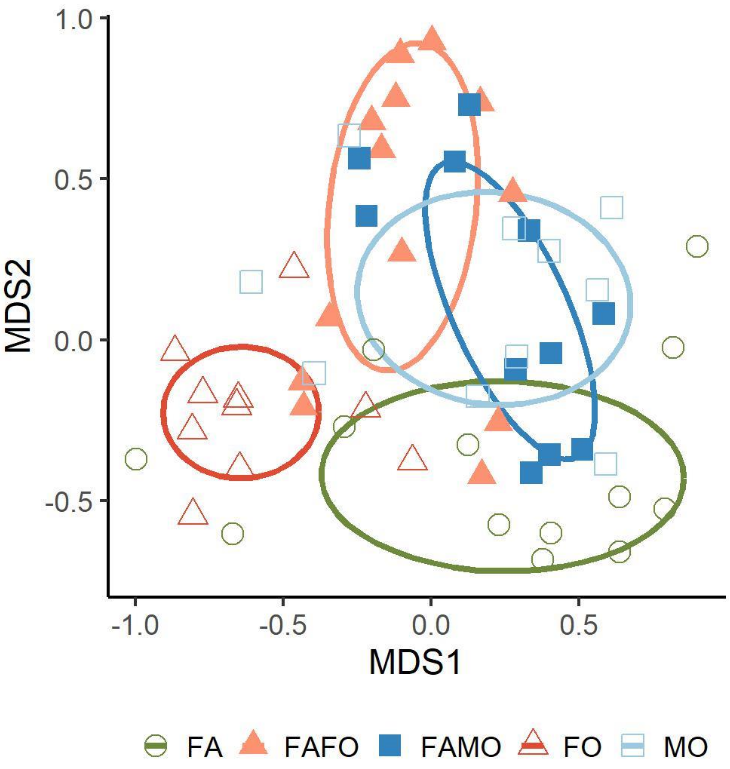

Figure 4 Non-metric multidimensional scaling (NMDS) plot illustrating the Bray-Curtis dissimilarities of the proportional volatiles compositions of wheat cultivars Florence-Aurora (FA) (n = 13), Forment (FO) (n = 10) and Montcada (MO) (n = 10) grown in monocultures and in mixtures: Florence-Aurora with Forment (FAFO) (n = 13) and Florence-Aurora with Montcada (FAMO) (n = 11).

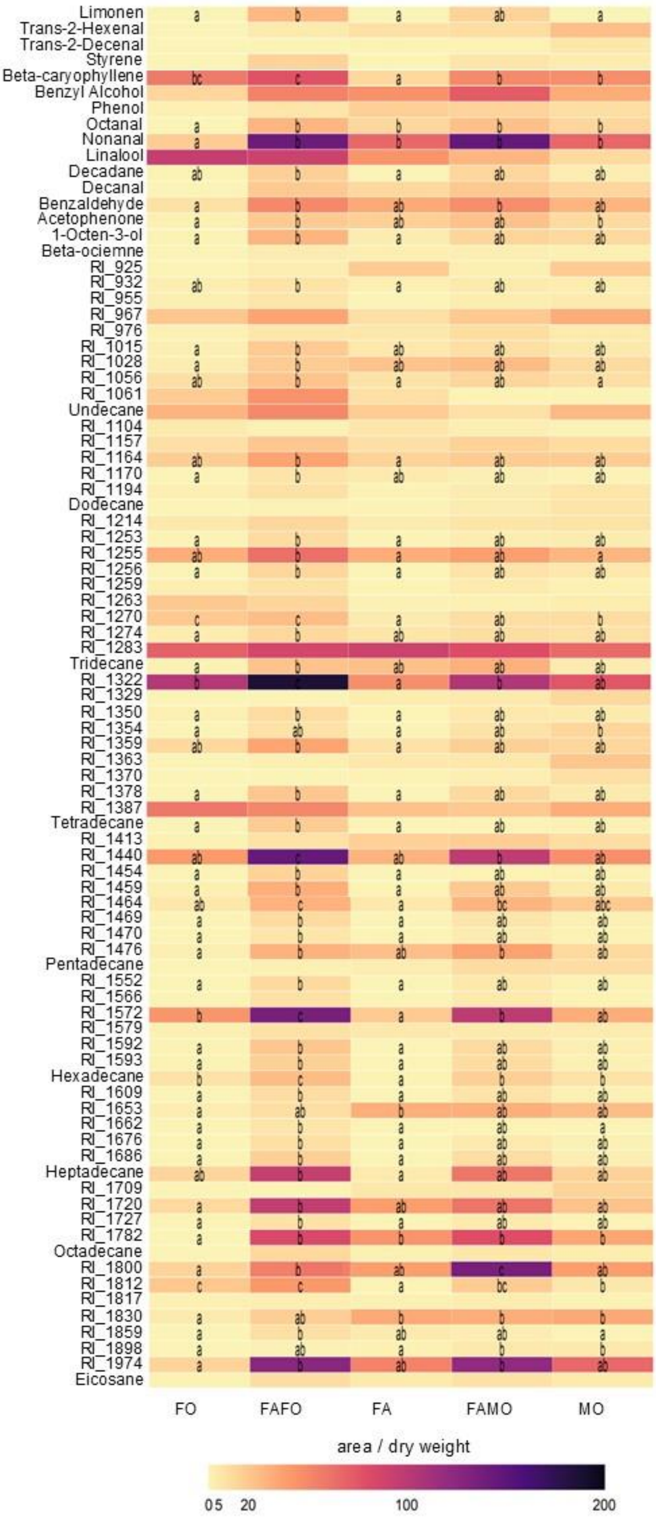

Figure 5 Composition of the volatile chemicals obtained by GC/SM of wheat treatments: Florence-Aurora (FA) (n = 13), Forment (FO) (n = 10), Montcada (MO) (n = 10), Florence-Aurora and Forment mixture (FAFO) (n = 13) and Florence-Aurora and Montcada mixture (FAMO) (n = 11). Dark blue indicates a high relative abundance of a respective volatile compound, light yellow a low abundance. Numbers are mean values of

compound abundance (compound peak area / dry weight (g)). Letters indicates significant differences according to the Dunn´s pairwise test ($P < 0.05$).

4. Discussion

4.1. Aphid olfactory response to cultivar mixtures

The purpose of this study was to assess whether combining wheat cultivars can modify the volatile mixture profile and, consequently, the aphid host-location response. Aphids are extremely sensitive to slight changes in their hosts' odor cues, which they utilize as host-finding signals (Webster 2012). Therefore, the alterations in mixture odor cues caused by plant-plant interactions can influence the aphid host-locating response and its attractiveness to particular wheat combinations, as demonstrated by our mixture of Florence-Aurora and Forment in olfactometer experiments with *Sitobion avenae*. Moreover, the volatiles emitted by Florence-Aurora with the formation mixture led to an avoidance by *Sitobion avenae*. Our results indicated that the Florence-Aurora and Forment mixture prevented aphid host localization by releasing non-attractive olfactory signals, thereby reducing aphid acceptance and further aphid infestation. Studies have demonstrated the importance of host-specific volatile compounds and their ratios in the overall composition of aphid host-location behavior (Webster 2012). For instance, regarding the volatile ratio in olfactometer tests reproducing hop (*Humulus lupulus*) leaf volatile cues, Campbell et al. (1993) found that aphids responded positively to the odor of (E)-2-hexenal and β-caryophyllene in an approximate ratio of 39:1 by weight. However, when the ratio was adjusted to 1:1, no response was observed. Therefore, a shift in the odor cues of Florence-Aurora and Forment cultivars when grown together may influence plant-pest interactions by decreasing crop odor attractiveness to aphids. In contrast, mixing Florence-Aurora and Montcada had no effect on the aphids' host-locating behavior compared to the monocultures or the odor mixture of the two cultivar monocultures, which is consistent with the absence of volatile profile alterations shown in the mixture.

4.2. Volatile emission of cultivar mixture

In this study, we assessed the interactions of undamaged wheat cultivars Florence-Aurora with Forment and Florence-Aurora with Montcada when grown together. The results showed that only certain cultivar combinations induced physiological responses to the volatiles emitted when grown together, suggesting the specificity of the effect of genotypic diversity on aphid control. Corroborating with previous experiments, headspace analysis revealed a higher amount of single volatile compounds released from the wheat cultivar mixture than from the monocultures (Shoffner & Tooker 2013).

Airborne volatiles are crucial signals for inter- and intraspecific plant-plant interactions. Plants constantly emit VOCs and, in return, are constantly exposed to VOCs from damaged and undamaged neighboring plants (Callaway 2002). These odor cues from emitter plants can affect complex biochemical pathways in the receiver plants (Midzi et al. 2022). Although most studies have focused on the induced defense response in plants receiving VOCs from herbivore-attacked plants (Hu et al. 2019; Midzi et al. 2022), previous studies have demonstrated that VOCs from undamaged plants also trigger morphological and physiological responses in receiver plants (Kheam et al. 2023; Ninkovic et al. 2016). For example, plants of the barley cultivar Kara (*Hordeum vulgare*) allocate more biomass to their roots after exposure to VOCs from cv. Alva compared to the unexposed plants, or cv. Kara plants previously exposed to VOCs of another Kara plant (Ninkovic et al. 2003)

We observed physiological responses in the form of altered release of VOCs by the Florence-Aurora and Forment cultivars when mixed. Furthermore, the analysis of specific compound amounts demonstrated that Florence-Aurora and Forment interactions, when grown together, altered the emitted amount of certain compounds, shifting the volatile ratio, which plays an important role in aphid host location (Webster 2012). Regarding specific volatile chemicals, Visser and Fu-Shun (1995) demonstrated that S. avenae was attracted to 2-hexanal, benzaldehyde, and linalool, but vaguely respond to 1-octen-3-ol, β-caryophyllene, and limonen odor signals. Moreover, 2-hexanal, linalool, octanal, nonanal, and caryophyllene are assumed to be strong cereal aphid attractants *Sitobion avenae* and *Rhopalosiphum padi* (Linnaeus) (Birkett et al. 2010; Pickett et al. 1997; Quiroz & Niemeyer 1998). In our study, the abundance of these volatiles was greatest in the Florence-Aurora and Forment mixture, whose odor cues were surprisingly less attractive than those of the monocultures. This suggests that the plant volatile ratio may play a more important role in modifying aphid behavior than the abundance of specific volatiles (Bruce & Pickett 2011).

Further, the analysis of the odor profile of single cultivars confirmed that the Florence-Aurora cultivar had a volatile profile similar to Montcada but significantly different from the Forment profile. The interaction between the Florence-Aurora and Montcada cultivars had no effect on the mixed odor profile. In line with previous volatile barley experiments, our findings supports the hypothesis that greater differences between cultivars odor profiles might induce greater physiological responses (Dahlin et al. 2018).

In conclusion, our study supports the "right neighbor" hypothesis by demonstrating an intraspecific interaction effect on the odor profile of mixtures, exclusive to certain cultivar mixtures, and might explain the dependence of genotypic diversity on aphid control recorded in numerous field studies (Dahlin et al. 2018; Ninkovic et al. 2002). Our results suggest that the similarity/dissimilarity of VOC emissions is important for plant-plant interactions between the combined cultivars. In the present study, wheat cultivars with distinct profiles affected each other, resulting in an odor that was less attractive to S. avenae. Future studies should address whether plants with generally dissimilar odor profiles are more likely to have an impact on each other, and thereby possibly promote associational resistance and enhance aphid control.

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Discussió general

La recerca en l'àmbit de l'agricultura ecològica ha assenyalat recentment la importància de la diversificació, des de l'escala de camp o finca fins a l'escala de paisatge, com a mecanisme per millorar la funcionalitat del sistema agrícola. Aquesta funcionalitat es refereix a la capacitat de la biodiversitat planificada i associada d'oferir serveis com el control de plagues, la regulació de les poblacions arvenses, la resistència a la sequera, l'aprofitament de recursos minerals i lumínics i la millora en la producció (Costanzo & Bàrberi 2014; Hatt & Döring 2023).

La investigació en cultius herbacis extensius ecològics també ha revelat que la diversificació en si mateixa no garanteix una major funcionalitat. És necessari comprendre la identitat i les interaccions entre les espècies i les varietats combinades, així com la influència dels factors ambientals, entre altres aspectes. En aquest sentit, encara queda molt per explorar sobre els beneficis i perjudicis dels policultius intraespecífics i interespecífics en els conreus herbacis extensius. Així doncs, la present tesi doctoral pretén avançar en el coneixement de la gestió agrícola ecològica en la producció de cereals de secà per a gra, en un context de clima mediterrani.

La discussió general de la tesi s'estructura en quatre seccions. En la primera secció s'avalua el paper de l'associació de varietats de blat d'hivern en el control de les poblacions d'espècies arvenses i de pugons mitjançant experiments realitzats en condicions naturals. A més, es discuteix la influència de les pràctiques de maneig del sòl en la funcionalitat dels policultius i també l'efecte de la introducció d'un cultiu de cobertura de lleguminosa en els monocultius i les associacions de varietats de blat. En la segona secció de la discussió s'exposen els mecanismes *bottom-up* relacionats amb el control de plagues de pugons en els cultius cerealistes. En aquesta secció es discuteixen els resultats dels quatre capítols que conformen la tesi doctoral. En la tercera secció s'expliquen els mecanismes *top-down* del control de plagues de pugons observats en els dos experiments en condicions naturals. Finalment, la quarta secció recull els resultats de l'anàlisi sobre la producció de blat en parcel·les en règim de monocultiu i de policultiu.

1. L'efecte de l'associació de varietats de blat sobre el control de les poblacions d'espècies arvenses i de pugons

Un dels principis de l'agricultura ecològica és la prohibició de l'ús d'aportacions externes, com ara els fitosanitaris (herbicides, insecticides, etc.). Això implica la necessitat de cercar estratègies alternatives per a la gestió d'herbes i plagues. Així doncs, és essencial potenciar l'establiment d'espècies i varietats de cereal que presentin la capacitat de competir amb les poblacions d'arvenses i siguin resistents a plagues. Per fer front aquest repte, un dels objectius de la tesi doctoral és avaluar l'eficàcia de diverses varietats de blat cultivades soles i associades en el control de les poblacions d'espècies arvenses i de pugons en condicions naturals. En aquesta tesi doctoral, l'abundància de les poblacions d'espècies arvenses s'ha estimat a partir de la biomassa per unitat de superfície (*capítol 1*), mentre que l'abundància de pugons s'ha analitzat a partir de la suma de pugons per parcel·la (c*apítols 1 i 2*).

Els nostres experiments en parcel·les experimentals permanents i camps comercials en una àrea agrícola dedicada als conreus herbacis extensius de secà mediterranis, posen de manifest que els monocultius de varietats modernes de blat (prenent com a referència la varietat Florence-Aurora) són molt susceptibles a la infestació de pugons i presenten una elevada abundància d'herbes. En canvi, els monocultius de les varietats tradicionals, Forment, Xeixa i Montcada, es caracteritzen per ser més competitives enfront de les poblacions arvenses i més resistents als pugons.

La comparació de l'abundància de les poblacions arvenses i de pugons entre parcel·les cultivades amb la barreja de varietats de blat modernes i tradicionals i parcel·les cultivades només amb les varietats modernes posa de manifest la millora en la prestació de serveis de regulació gràcies a les funcions ecològiques intrínseques de les varietats tradicionals. La barreja de les tres varietats de blat Florence-Aurora, Forment i Xeixa, comporta la disminució de la biomassa de les poblacions d'espècies arvenses en condicions d'alta infestació i la reducció de l'abundància de pugons en comparació amb el monocultiu de la varietat més moderna. La menor abundància de pugons també s'ha observat en l'associació de Florence-Aurora amb Forment.

Els beneficis de la barreja de les tres varietats de blat en la regulació de les poblacions d'espècies arvenses es podria explicar l'eficiència més gran en l'ocupació de l'espai aeri gràcies a la complementarietat de les diferents alçades de les tres varietats. Dit d'una altra manera, la combinació de varietats tradicionals de port alt, bones competidores pels recursos lumínics i nutricionals, amb varietats de port més baix incrementaria la capacitat competitiva del cultiu davant les espècies arvenses (Worthington et al. 2013; Lazzaro et al. 2018) (*capítol 1*). A més, la major complexitat de l'estructura aèria de la barreja de blats també pot afavorir al control de plagues. Les varietats de port alt actuen com una barrera física que dificulta la mobilitat dels insectes i alhora interfereix en la localització visual de les varietats modernes de port baix, que sovint són més susceptibles a les plagues (Finch & Collier 2000; Ficiciyan et al. 2018) (*capítol 1-2*).

La funcionalitat dels policultius de blat ve condicionada per la identitat i la interacció entre les varietats combinades tal com exposa la hipòtesi del bon veí (Ninkovic et al. 2002; Dahlin et al. 2018). La combinació de dues varietats morfològicament i fisiològicament similars, com la barreja de Florence-Aurora amb Montcada, ha estat poc eficaç en el control de les poblacions de pugons; només ha estat beneficiosa durant el període d'alta infestació de pugons la primavera de 2021. En canvi, la combinació de varietats amb trets funcionals diferents i complementaris (l'alçada, la data d'espigat, el contingut en nitrogen tissular i el perfil olfactiu), com la barreja de Florence-Aurora amb Forment i Xeixa, ha estat clau per incentivar el control de les poblacions de pugons i la regulació de poblacions d'espècies arvenses.

Els resultats obtinguts en els diversos experiments d'aquesta tesi doctoral són notables si es comparen amb investigacions anteriors que no han considerat els atributs funcionals de les varietats a l'hora de dissenyar l'experimentació. Per exemple, a l'estudi de Lazzaro et al. (2018) les associacions de tres i sis varietats de blat no provocaven una reducció en la biomassa de les poblacions arvenses, que va ser similar a la de les varietats en monocultiu. Pel que fa al control de les poblacions de pugons, treballs previs en condicions naturals mostren un efecte neutre del policultiu de blats (Koricheva & Hayes 2018; Mansion-Vaquié et al. 2019) mentre que estudis en hivernacle han evidenciat la disminució de les poblacions de pugons quan s'associen tres o més varietats de blat (Shoffner & Tooker 2013; Grettenberger & Tooker 2016).

1.1 La influència de les pràctiques de maneig del sòl sobre la funcionalitat dels sistemes agrícoles

Un aspecte a destacar de la tesi doctoral és l'anàlisi de la incidència de la intensitat de la llaurada (l'arada de pales respecte de l'arada de cisell) i de la fertilització (la periòdica incorporació de fems compostats respecte a la no incorporació de fems) sobre l'abundància de pugons, l'abundància de les poblacions d'espècies arvenses i la producció de gra en una associació de tres varietats de blat, Florence-Aurora, Forment i Xeixa i en el monocultiu de cadascuna de les varietats en una parcel·la experimental permanent situada a l'EIN de Gallecs (*capítol 1)*.

El nostre estudi posa de manifest que l'abundància de les poblacions arvenses, estimada a partir de la biomassa aèria, és el 22,14% l'any 2019 i el 19,89% l'any 2021 menor a les parcel·les llaurades amb l'arada de pales que a les llaurades amb l'arada de cisells a causa de la major intensitat de pertorbació del sòl. Aquest patró ja va ser assenyalat per Baldivieso-Freitas (2018) durant els primers quatre anys de la rotació en el mateix experiment. No obstant això, l'anàlisi de la interacció entre la intensitat de llaurada i el tipus de cultiu reflecteix que la biomassa d'herbes en les parcel·les amb arada de pales no varia significativament entre tractaments de blat. En canvi, a les parcel·les sotmeses a la llaurada de cisell, amb major abundància d'herbes, els monocultius de varietats tradicionals de port alt (Forment i Xeixa) i la triple barreja afavoreixen la regulació de poblacions arvenses respecte al monocultiu de Florence-Aurora.

Pel que fa al control de les poblacions de pugons, les parcel·les llaurades amb l'arada de pales han presentat una major infestació de pugons. Possiblement, perquè la reducció de l'abundància i la diversitat d'espècies arvenses repercuteix negativament sobre l'atracció de la fauna auxiliar beneficiosa (Hatt et al. 2018). Tot i que en condicions de llaurada amb arada de pales hi hagi major incidència de pugons, els monocultius de varietats tradicionals Xeixa i Forment i la triple barreja han mostrat menor població de fitòfags que el monocultiu de Florence-Aurora. Finalment, la fertilització nitrogenada no ha provocat cap efecte en la població de pugons.

Així doncs, els resultats del nostre estudi demostren que la gestió del sòl influeix en les condicions biòtiques locals com és l'abundància de les poblacions arvenses que, al seu torn, afecta la regulació de les poblacions de pugons del cultiu de cereals. Per tant, per tal de garantir la prestació dels serveis agroecosistèmics desitjats, cal tenir en compte la resposta dels policultius de blat a les pràctiques agrícoles utilitzades en el conreu.

1.2 Els beneficis i perjudicis sobre el control de plagues en incorporar una coberta de melgó en monocultius i barreges de varietats de blat

En el *capítol 2*, s'ha valorat l'efecte d'incorporar als monocultius de les varietats Florence-Aurora, Montcada i Forment i a les dues associacions de varietats de blat (Florence-Aurora amb Montcada i Florence-Aurora amb Forment) una coberta vegetal d'una lleguminosa anual, concretament el melgó, sobre el control de plagues. L'ús de la coberta de melgó ha demostrat ser efectiu en el control de plagues especialment quan s'associa als monocultius de varietats de blat sensibles als pugons, com Florence-Aurora. Atès que els pugons són fitòfags especialitzats, la sembra simultània d'una coberta que no sigui de cereal, oculta els senyals visuals i olfactius del blat (Tahvanainen & Root 1972). A més, les cobertes vegetals poden obstaculitzar físicament la propagació dels pugons (Mansion-Vaquié et al. 2017). No obstant això, contràriament a l'esperat, els policultius de blat amb coberta de melgó no han contribuït a augmentar l'abundància i riquesa de la fauna auxiliar útil, artròpodes predadors aeris i caminadors, així com vespes parasitoides (Lopes et al. 2016; Hatt et al. 2018).

La combinació de dues pràctiques com són l'associació de la barreja de varietats de blat i la incorporació de la coberta de melgó no ha millorat la regulació de les poblacions de pugons respecte a l'ús d'una sola pràctica. Tant la barreja de Florence-Aurora amb Forment com el monocultiu de Florence-Aurora amb coberta de melgó, han afavorit la resistència associativa mitjançant mecanismes de regulació *bottom-up.* Per tant, la seva combinació, Florence-Aurora amb Forment més coberta de melgó, no ha implicat un benefici addicional per la regulació de les poblacions de pugons atès a la manca de complementarietat entre les funcions ofertes, és a dir són pràctiques funcionalment redundants (Barbosa et al. 2009; Gaba et al. 2015; Barot et al. 2017).

2. Mecanismes de control *bottom-up* lligats a l'associació de varietats de blat

En els estudis en condicions naturals, s'ha comparat el nombre de pugons per fillol d'una mateixa varietat cultivada en monocultiu i policultius (barreja de tres varietats Florence-Aurora, Xeixa i Forment, barreja de dues varietats Florence-Aurora amb Montcada i Florence-Aurora amb Forment i la incorporació de la coberta de melgó) per tal d'evidenciar els mecanismes *bottom-up* involucrats en el control de plagues (*capítols 1-2*). Els experiments mostren un menor nombre de pugons en les plantes de Florence-Aurora, una varietat molt susceptible als pugons, quan és associada a varietats de blat resistents i amb trets complementaris (Xeixa i Forment o únicament amb Forment) i quan s'incorpora una coberta de melgó (*capítol 1-2)*. Aquests resultats es poden explicar per dos mecanismes. El primer es basa en el fet que la sembra conjunta de varietats resistents i susceptibles, o bé la incorporació al cultiu d'una espècie no-hoste, dificulta la localització de la varietat més sensible per part dels pugons. El segon es recolza amb el fet que les interaccions planta-planta presents en els policultius poden haver causat modificacions morfològiques o fisiològiques que hagin incrementat la resistència de les varietats més susceptibles (Barbosa et al. 2009; Dahlin et al. 2020)

En ambdós casos, els nostres resultats suggereixen relacions de facilitació entre les varietats sensibles i resistents, així com entre espècies que ha reforçat la resistència associativa del policultiu.

2.1 Identificació de trets funcionals lligats a la resistència associativa de les varietats de blat

Paral·lelament als estudis en condicions naturals, s'han dut a terme dues experiències complementàries en ambients controlats d'hivernacle i de laboratori amb la finalitat d'aprofundir en els mecanismes *bottom-up* observats en condicions naturals. Per aquest motiu, s'han caracteritzat tres trets funcionals, el perfil olfactiu (*capítol 4*), el contingut de nitrogen tissular i la biomassa aèria (*capítol 3)* de les varietats Florence-Aurora, Montcada i Forment sembrades en monocultiu i en associació dos a dos (Florence-Aurora amb Montcada i Florence-Aurora amb Forment). La raó per la qual s'han analitzat aquests atributs es fonamenta en el fet que el perfil olfactiu juga un paper molt important en la fase de localització de l'hoste (Webster 2012; Ninkovic et al. 2016), Així mateix, el nitrogen tissular del blat i la biomassa aèria estan relacionats amb la qualitat nutricional de l'hoste i el creixement de les poblacions (o colònies) de pugons (Aqueel & Leather 2011) Aquests experiments han utilitzat les mateixes varietats en monocultiu i les mateixes associacions de blat que les establertes en condicions naturals en el segon capítol de la tesi.

La caracterització del perfil olfactiu de les diferents varietats de blat cultivades soles o associades s'ha dut a terme amb la tècnica de cromatografia de gasos. Cadascuna de les plantes emprades en l'experiment es van fer créixer aïllades dels senyals olfactius de la resta de plantes per evitar contaminacions. L'aïllament es va aconseguir col·locant les mostres dins de caixes de Perspex connectades a una bomba d'aire que enviava l'aire de dins la caixa a l'exterior (Glinwood et al. 2009) (*capítol 4*). L'anàlisi del contingut de nitrogen tissular es va realitzar amb la tècnica de la combustió de Dumas (*capítol 3).*

Els resultats han revelat l'heterogeneïtat de perfils olfactius, de contingut de nitrogen tissular i de biomassa aèria entre les tres varietats de blat analitzades. Les varietats Florence-Aurora i Forment han presentat perfils olfactius diferents i Forment ha mostrat un contingut de nitrogen tissular i una biomassa menor que les altres dues varietats. Aquesta heterogeneïtat és essencial per entendre la resistència associativa de les barreges varietals de cereals, ja que, suggereix la possibilitat de combinar varietats amb mecanismes de resistència complementaris (Barot et al. 2017).

El perfil olfactiu i el contingut de nitrogen de les plantes de Florence-Aurora i Montcada crescudes en policultiu no ha mostrat dissimilituds amb aquelles plantes crescudes en monocultiu. Així doncs, la combinació d'aquestes varietats difícilment serà més resistent als pugons que els monocultius que la conformen. A més, les anàlisis a l'hivernacle han demostrat que l'associació de Florence-Aurora i Montcada causa un augment de la biomassa mitjana de les plantes de blat. Una major biomassa permet sostenir una major població de pugons (Szpeiner et al. 2009).

En canvi, quan s'han associat varietats amb olors diferents com són Florence-Aurora i Forment, la barreja ha presentat un perfil olfactiu distint al dels monocultius. Els perfils olfactius dels monocultius de Florence-Aurora i Forment i el perfil de la barreja difereixen en la quantitat emesa d'alguns volàtils, és a dir, no ha canviat la composició, sinó la proporció dels volàtils emesos. A més, el contingut de nitrogen tissular de les plantes de Florence-Aurora va ser menor quan creixen associades amb el Forment.

Els nostres resultats concorden amb estudis anteriors on la interacció entre determinades varietats d'ordi afectava a atributs com l'alçada, la fenologia i l'índex d'àrea foliar (Dahlin et al. 2020). El nostre estudi fa un pas més enllà i suggereix que les alteracions en els atributs es produeixen principalment per la combinació d'aquelles varietats que presentin diferències en el tret funcional analitzat. Finalment, tal com s'ha mencionat anteriorment, aquestes alteracions podrien repercutir positivament en la resistència associativa del policultiu (*capítol 3-4*).

2.2 Estudi de la interacció planta-planta i planta-pugó en experiments d'hivernacle i laboratori

De manera simultània a la caracterització dels trets funcionals de les varietats de blat, s'ha avaluat les interaccions planta-planta i planta-pugó (*capítols* 3 i 4). Per l'anàlisi de la interacció planta-pugó es va utilitzar una població de *Sitobion avenae,* ja que va ser l'espècie més abundant al camp. L'experimentació en hivernacle i laboratori permet uniformitzar els factors ambientals, ecològics i agronòmics i així aprofundir en el paper de la resistència associativa en diferents fases de selecció de l'hoste i del desenvolupament de la població de pugons.

Fase de localització de l'hoste

La interacció planta-pugó en la fase de localització de l'hoste s'ha avaluat mitjançant l'ús d'olfactòmetres (Glinwood et al. 2009). Aquest dispositiu s'utilitza per estudiar el comportament dels insectes en presència de dos estímuls olfactius procedents de dos tractaments que es comparen simultàniament. Així doncs, amb aquest estudi s'obtenen preferències relatives entre tractaments en comptes de resultats absoluts. L'olfactòmetre també permet testar la resposta de rebuig dels pugons als senyals olfactius dels tractaments a partir de la comparació entre els estímuls olfactius d'un tractament vers el control negatiu (aire net) (*capítol 4*).

Els resultats mostren, en primer lloc, que la preferència dels pugons no varia entre els perfils olfactius de les tres varietats cultivades en monocultiu. Per tant, la major susceptibilitat de Florence-Aurora no està relacionada amb l'emissió de senyals olfactius més atractius. En segon lloc, els pugons no tenen una clara preferència entre els senyals olfactius de la barreja de Florence-Aurora i Montcada i els dels respectius monocultius, probablement perquè els tres tractaments presenten perfils olfactius similars.

En canvi, els pugons prefereixen els senyals olfactius de Florence-Aurora respecte a l'olor de la barreja de Florence-Aurora amb Forment i fins i tot, els pugons mostren una clara preferència per la suma dels senyals olfactius de Florence-Aurora i Forment cultivats en condicions de monocultiu respecte als senyals emesos per la barreja. Per obtenir la suma de senyals olfactius de dos monocultius, es connecten simultàniament els tractaments Florence-Aurora i Forment a una de les bandes de l'olfactòmetre, així doncs, el pugó respon a la combinació de les olors dels dos monocultius. Per equiparar el volum de senyals olfactius emesos entre els tractaments comparats, cal col·locar dues mostres de la barreja Florence-Aurora amb Forment a l'altra banda del dispositiu. A més, els pugons van mostrar comportament de rebuig únicament cap als senyals olfactius de l'associació de Florence-Aurora i Forment.

Els resultats d'aquest experiment mostren, per una banda, que determinades associacions de varietats de blat poden afavorir la resistència associativa mitjançant l'emissió de senyals olfactius poc atractius, fins i tot repulsius, els quals interfereixen negativament en el procés de localització i selecció de l'hoste. Per altra banda, si tenim en compte que els perfils olfactius dels monocultius Florence-Aurora, Forment i la barreja d'aquestes dues varietats, només difereixen en la proporció de volàtils, s'arriba a la conclusió que els pugons tenen una alta sensibilitat olfactiva capaç de detectar i respondre a aquesta variació. Així, per exemple, Campbell et al. (1993) van demostrar que els pugons eren atrets per les plantes de llúpol amb un perfil olfactiu format per dos compostos volàtils, (E)-2-hexenal i β-cariofil·lè emesos en una proporció aproximada de 39:1 en pes, però quan la proporció es va ajustar a 1:1, no es va observar cap resposta dels pugons.

Fase acceptació de l'hoste

La fase d'acceptació de l'hoste s'ha analitzat a partir del número de pugons fixats a una planta de blat després de 2 h d'establir contacte. Les plantes utilitzades en aquest test havien estat exposades prèviament durant una setmana als compostos volàtils de Florence-Aurora, Montcada o Forment.

Els resultats de l'experiment evidencien que l'acceptació dels pugons per les plantes de Forment és menor quan han estat exposades als volàtils de Florence-Aurora (*capítol 3*).
Aquests resultats són coherents amb els treballs fets per Ninkovic et al. (2002), els quals indiquen que la interacció entre plantes a través de volàtils influeix en el procés de selecció de l'hoste. Aquesta disminució en l'acceptació pot ser el resultat d'alteracions en els volàtils emesos, com hem constatat amb l'anàlisi dels perfils olfactius, o bé per canvis en el tacte o en la qualitat nutricional de la planta. És important destacar que aquesta resposta és, novament, específica de les interaccions entre varietats de blat amb senyals olfactius diferents. Tanmateix, és rellevant assenyalar que la resposta ha estat unidireccional, és a dir, l'acceptació de la varietat Forment disminueix quan ha estat exposada a Florence-Aurora, però no al revés.

Fase desenvolupament de la colònia de pugons

La fase del desenvolupament de la colònia de pugons s'ha estimat a partir del creixement de la colònia de pugons en quatre plantes de blat cultivades juntes en un test i partir del número de pugons per planta de blat. Les dues mesures s'han avaluat en els cinc tractaments de blat establerts (els tres monocultius de Florence-Aurora, Montcada i Forment i les dues barreges de Florence-Aurora amb Montcada i Forment respectivament). De manera similar als resultats obtinguts en condicions naturals, l'associació de Florence-Aurora amb Forment conté una menor població de pugons respecte al monocultiu de Florence-Aurora i les plantes crescudes en aquesta barreja presenten menys abundància de pugons per individu. Per tant, s'evidencia que la resistència associativa dels policultius intraspecífics de cereal és derivada de mecanismes *bottom-up*, és a dir, de mecanismes de control de plagues oferts per nivells tròfics inferiors. Contràriament el que s'esperava, la correlació entre el contingut de nitrogen tissular i el creixement de la colònia de pugons ha estat baixa (*capítol 3*). Així doncs, s'hauran d'analitzar altres trets de qualitat com la composició del floema (aminoàcids, sucrosa, àcids orgànics) que podrien tenir major rellevància pels organismes fitòfags (Gallinger & Gross 2020).

D'aquestes experiències al laboratori i hivernacle, se'n desprenen tres idees principals. En primer lloc, que els fenòmens de resistència associativa vinculats a mecanismes *bottom-up* estan presents en diferents fases del procés de selecció de l'hoste i desenvolupament de la colònia de pugons (*capítol 3-4)*. En segon lloc, que la resistència associativa observada, tant en condicions naturals com d'hivernacle, en la barreja de Florence-Aurora amb Forment s'explica per fenòmens relacionats amb la localització i la qualitat de l'hoste, que dificulten la infestació i el posterior creixement de les poblacions de pugons (*capítol 2-4)*. En tercer lloc, els nostres estudis semblen suggerir que la combinació de varietats amb trets funcionals diferents afavoreix la resistència associativa envers les poblacions de pugons (*capítol 2-4*).

3. Mecanismes de control *top-down* relacionats amb l'associació de varietats de blat

Els efectes dels mecanismes *top-down* s'han analitzat de manera directa, mitjançant la taxa de parasitisme (c*apítol 1 i* 2) i de manera indirecta a partir de l'abundància de les poblacions de predadors voladors i caminadors (*capítol 2*) en experiments de camp. Tant la taxa de parasitisme com l'abundància de predadors voladors (coccinèl·lids, aràcnids i larves de sírfids) s'ha obtingut a partir de recomptes visuals en mostrejos lineals. En canvi, els predadors caminadors (aràcnids, caràbids i estafilínids) s'han recol·lectat amb trampes de caiguda.

La taxa de parasitisme ha mostrat valors baixos en els dos estudis. El valor màxim registrat ha estat del 22,4 ± 1,3% (mitjana ± ES) en el monocultiu de Florence-Aurora en la parcel·la experimental permanent durant el primer any d'experimentació.

Els nostres resultats assenyalen que la diversificació en camps de blat ha tingut poca repercussió sobre el parasitisme, ja que no s'han detectat diferències significatives entre els diferents tractaments de varietats de cultius, o bé, aquestes diferències no han estat consistents entre els períodes de mostreig. A més, no s'ha observat una clara relació entre l'abundància de pugons i la taxa de parasitisme (Mansion-Vaquié et al. 2019). Aquests resultats coincideixen amb la metaanàlisi realitzada per Koricheva & Hayes (2018), que demostra la baixa relació entre la diversitat planificada i l'abundància i diversitat de parasitoides, així com la taxa de parasitisme.

Els artròpodes predadors caminadors més abundants han estat les aranyes (58,3%) i els estafilínids (32,3%), dos grups de predadors generalistes. En el cas dels predadors aeris, es van compatibilitzar principalment predadors especialistes com els coccinèl·lids en estadi larvari i adult (57,0%). De manera semblant amb investigacions anteriors, la major complexitat aportada per les associacions de varietats de blat no ha afavorit ni a l'abundància ni a la riquesa d'artròpodes predadors, ni caminadors ni aeris (Lopes et al. 2016; Mansion-Vaquié et al. 2017). Una possible interpretació seria que les barreges de varietats de cereal no ofereixen recursos nutricionals secundaris (per exemple pol·len i nèctar) i que la complexitat aèria que en resulta no és suficient per atreure la fauna auxiliar beneficiosa.

En resum, els experiments establerts en condicions naturals han posat de manifest que els policultius de varietats de blat tenen poca incidència sobre la fauna auxiliar beneficiosa i, per tant, és poc probable que el control de plagues observat en els policultius sigui fruit de mecanismes *top-down.* Per la qual cosa, es reforça la idea que els mecanismes *bottom-up* són fonamentals per la funció de control de plagues en policultius intraespecífics de blat.

Finalment, els resultats suggereixen que altres factors no considerats en l'estudi poden tenir una influència major en la taxa de parasitisme i en la comunitat d'artròpodes predadors caminadors i aeris. D'acord amb investigacions anteriors, la taxa de parasitisme en cultius herbacis extensius sembla estar condicionada per la presència de marges florals anuals i marges llenyosos, com les bardisses, més que per les estratègies de diversificació dins del cultiu (Dassou & Tixier 2016; Salat-Moltó et al. 2023). A més, l'estructura i composició del paisatge té un impacte en l'abundància de coccinèl·lids i caràbids (Caballero-López et al. 2012). Així doncs, cal considerar altres estratègies de diversificació, com la incorporació de bandes florals o el restabliment i la conservació dels marges arbustius i arboris, per promoure la presència de fauna auxiliar beneficiosa.

4. L'efecte de l'associació de varietats de blat sobre la producció del cultiu

Un dels aspectes més important a considerar pels agricultors a l'hora d'implementar els policultius a la seva finca són els relacionats amb les possibles pèrdues de producció (Di Bene et al. 2022). És per aquest motiu, que en els experiments en condicions naturals s'ha comparat la producció del gra (kg/ha) dels diferents monocultius amb els policultius de blat (c*apítol 1 i 2)*. A més, per al disseny experimental, s'ha tingut en compte la viabilitat comercial de les barreges. Per això, s'han combinat varietats de blat que es puguin processar conjuntament per l'elaboració de farina panificable d'alta qualitat. També es va tenir en compte la fenologia de les diferents varietats de blat amb la intenció de fer una collita simultània de les varietats barrejades (*capítol 1)*. L'anàlisi de la fenologia de les varietats indica que Florence-Aurora és una varietat primerenca, amb una data d'espigat de tres a quatre setmanes abans que Xeixa i el Forment. Però el decalatge fenològic en el moment de la floració, disminueix durant la maduració del gra, fins al punt que aquesta varia en un rang de deu dies. Aquesta sincronització facilita la gestió agrícola de la barreja, i concretament la recol·lecció del gra (Newton et al. 2010).

Estudis anteriors han demostrat que les varietats modernes de cereals tenen una producció de gra superior a les tradicionals, especialment en condicions de baixa competitivitat amb les poblacions d'espècies arvenses (Kaut et al. 2008; Lazzaro et al. 2018). No obstant això, els nostres estudis mostren una producció significativament similar entre els monocultius de les varietats més modernes i les tradicionals. Al mateix temps, els diversos policultius de varietats i la incorporació de la coberta de melgó, tampoc han influenciat de manera significativa la producció del gra.

De manera excepcional, la producció del monocultiu modern de Florence-Aurora va ser molt baixa la campanya de 2021 a causa de les condicions ambientals adverses, ja que la pluviometria a l'àrea de Gallecs va ser un 80% inferior a la mitjana anual, mentre que els monocultius de les varietats tradicionals i els policultius van mostrar una producció comparable a la dels anys anteriors. D'aquests resultats se'n desprèn que els policultius són més estables enfront dels possibles canvis ambientals, gràcies a la resiliència que ofereixen les varietats tradicionals (Reiss & Drinkwater 2018). Així doncs, la barreja de varietats més modernes amb tradicionals podria ser un mecanisme per esmorteir les possibles pèrdues de producció dels anys desfavorables.

La incorporació de la coberta de melgó no ha tingut cap efecte sobre la producció de gra del blat. L'altura mitjana del melgó, que varia entre 10 i 50 cm, és més baixa que l'alçada de les varietats cultivades, i per això es podria assumir que la incorporació de melgó als cultius de blat no genera competència pels recursos lumínics. Així mateix, s'esperaria que el melgó millorés la producció de blat gràcies a la fixació de nitrogen atmosfèric característic de les lleguminoses (Stagnari et al. 2017). Malauradament, l'efecte de l'ús d'una coberta sobre el rendiment del cultiu només es va poder avaluar el primer any de mostreig a causa del deficient establiment el segon any, temps insuficient perquè el nitrogen fixat estigués disponible pel cultiu.

L'estudi de l'efecte de la intensitat de la llaurada i la fertilització sobre la producció de gra de la triple barreja de Florence-Aurora, Xeixa i Forment i els respectius monocultius en la parcel·la permanent de l'EIN de Gallecs (*capítol 1*) posa de manifest que les pràctiques del maneig del sòl condicionen la producció dels cultius.

En concordança amb investigacions anteriors a la mateixa parcel·la, la fertilització ha estat el factor que més afecta la producció de gra (Tamburini et al.; Baldivieso-Freitas et al. 2018). La producció de gra de blat ha disminuït de manera significativa a les subparcel·les sense fertilització tant en el monocultiu de la varietat moderna com els monocultius de les varietats tradicionals, així com en la triple barreja Florence-Aurora amb Xeixa i Forment. Això s'explicaria perquè els cereals tenen una eficiència baixa en l'ús del nitrogen i, en conseqüència el nitrogen esdevé un nutrient limitant pel seu desenvolupament (Diacono et al. 2019).

L'efecte de la intensitat de llaurada sobre la producció ha variat amb relació al tipus de cultiu. La producció de gra de blat ha disminuït de manera significativa a les subparcel·les gestionades amb l'arada de cisell en el monocultiu de Florence-Aurora i en la triple barreja. Aquest fet es podria explicar per la major abundància de les poblacions arvenses que competeixen pels recursos amb el cultiu (Kaut et al. 2008; Lazzaro et al. 2018), i per la menor disponibilitat de nitrogen a causa del retard en la mineralització, ambdós aspectes relacionats amb la reducció de la pertorbació del sòl (Mäder & Berner 2012). En canvi, la producció dels monocultius de les varietats tradicionals, Xeixa i Forment, no s'ha vist afectada per la intensitat llaurada, ja que en ser varietats de port alt són més bones competidores i han disminuït població les poblacions d'espècies arvenses fins i tot en subparcel·les llaurades amb arada de cisell (*capítol 1*).

Conclusions

En aquesta tesi doctoral s'ha avaluat l'efecte de l'increment de diversitat agrícola planificada (barreja de varietats de blat, ús d'una coberta vegetal de melgó (*Medicago polymorpha*) i l'associació d'ambdues) sobre la funcionalitat dels conreus ecològics de blat d'hivern. Els estudis s'han dut a terme mitjançant la combinació d'experiments en condicions naturals durant dos anys i diverses experiències d'hivernacle i laboratori. Les varietats estudiades han estat Florence-Aurora, varietat moderna, les tradicionals Montcada, Xeixa (*Triticum aestivum* L. subsp. *aestivum*) i Forment (*Triticum turgidum* L. subsp. *durum*).

La funcionalitat del cultiu com ara la regulació de les poblacions d'espècies arvenses s'ha estimat a partir de l'anàlisi de la riquesa i biomassa de les poblacions arvenses en camps comercials. El control de plagues a partir de l'abundància total de pugons a les parcel·les i el nombre de pugons per fillol, i també la taxa de parasitisme i l'abundància i la riquesa d'artròpodes predadors dels pugons, i finalment, la producció del cultiu de blat.

Els experiments en condicions naturals també han permès avaluar la influència de la llaurada i la fertilització sobre la funcionalitat de l'associació de tres varietats de blat (Florence-Aurora amb Xeixa i Forment) i els seus monocultius. També s'ha estudiat la resistència associativa de barreges de varietats amb trets morfològics i fenològics similars (Florence-Aurora amb Montcada) o diferents (Florence-Aurora amb Forment), en comparació amb els respectius monocultius i l'associació d'una coberta vegetal de melgó en els cultius anteriors. En aquest últim experiment s'ha aprofundit en els mecanismes de control *bottom-up* i *top-down* vinculats amb la resistència dels policultius.

Els estudis en condicions controlades d'hivernacle i laboratori han permès abordar la relació planta-planta i planta-pugó, així com el paper del perfil olfactiu i la concentració de nitrogen tissular en el control dels pugons en les associacions de blat en diferents fases de la infestació, com la localització i la selecció de l'hoste i el desenvolupament posterior de la població de pugons.

A continuació s'exposen les principals conclusions recollides dels diferents capítols que conformen la tesi doctoral.

1. Les pràctiques de gestió del sòl influeixen en les poblacions d'espècies arvenses, en les poblacions de pugons i en la producció del cultiu de blat. Específicament, l'arada de pales ha disminuït la infestació d'herbes i ha incrementat l'abundància de pugons, i tant la llaurada de cisell com l'absència de fertilització han reduït la producció del monocultiu de Florence-Aurora i de la barreja de Florence-Aurora, Xeixa i Forment.

2. L'associació de Florence-Aurora amb Xeixa i Forment regula les poblacions d'espècies arvenses en condicions d'alta infestació d'herbes gràcies al fet que les varietats tradicionals, Xeixa i Forment, tenen un port més alt i, per tant, són més competitives.

3. La capacitat de les barreges de blat de regular les poblacions de pugons depèn de la identitat de les varietats combinades. Aquest estudi també suggereix que la resistència associativa és

més probable en barrejar varietats amb trets com l'alçada, perfil olfactiu, biomassa aèria o contingut de nitrogen en teixit vegetal diferents, com s'evidencia en Florence-Aurora i Forment. En canvi, la combinació de varietats amb trets funcionals similars, com són Florence-Aurora i Montcada, són menys beneficioses a causa de la redundància funcional.

4. Els mecanismes *top-down* tenen una baixa influència en el control de plagues en policultius de cereals perquè l'abundància i riquesa d'artròpodes predadors ha estat similar a la dels monocultius i, a més, no s'ha observat una clara relació entre la taxa de parasitisme i la població de pugons. Així doncs, el control de la població de pugons observada en experiments en condicions naturals i en hivernacle ha estat causat per mecanismes *bottom-up.*

5. La incorporació de la coberta de melgó a la barreja Florence-Aurora amb Forment no representa un benefici addicional per a la regulació de les poblacions de pugons respecte a la barreja sense la coberta, ja que ambdues pràctiques afavoreixen el control de plagues mitjançant mecanismes *bottom-up*. Per tant, és important conèixer les funcions de les espècies i varietats associades per evitar redundància funcional.

6. La resistència associativa de les barreges de blat comença en la fase de localització i selecció de l'hoste, perquè els pugons diferencien i seleccionen les varietats a partir de l'olor emesa. En aquesta línia, l'associació de varietats amb diferent perfil olfactiu, com són Florence-Aurora i Forment, modifica el perfil olfactiu de la barreja i l'olor emès resulta menys atractiu pels pugons que el dels monocultius.

7. Les varietats de blat Florence-Aurora, Forment i Montcada difereixen en el contingut de nitrogen tissular, amb Forment com la varietat amb menor contingut. La combinació de varietats amb contingut de nitrogen diferent, Florence-Aurora i Forment, ha disminuït el nitrogen tissular de Florence-Aurora. No obstant això, en l'experiment realitzat en hivernacle no s'ha observat una clara relació entre el contingut de nitrogen tissular i el creixement de la població de pugons.

8. La producció de gra ha sigut similar entre els monocultius i policultius de blat. Convé destacar, però que la producció del monocultiu Florence-Aurora ha variat significativament entre anys. En canvi, l'associació d'aquesta varietat amb varietats tradicionals, Xeixa, Forment i Montcada, ha estabilitzat la producció del cultiu entre els anys de mostreig.

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Thesis summary

Introduction

Agricultural ecosystems have been modified over time to produce food, fibers, forage, and more recently, biofuels. In this regard, in the middle of the 20th century agriculture underwent significant intensification, known as the "Green Revolution," aimed at increasing food yield to meet the needs of a constantly growing population (Tilman et al. 2002).

Agricultural intensification in cereal crops primarily involved the expansion of monocultures and the replacement of traditional cultivars with a few modern ones (Ficiciyan et al. 2018). These modern cultivars have been originated by breeding techniques to increase yield. However, they are highly susceptible to pests, diseases and weed infestation. In turn, the establishment of modern cultivars monocultures significantly increased the use of pesticides for pest control, intensive tillage practices for weed control, as well as the application of nitrogen and phosphorus chemical fertilization (Tilman et al. 2002). Moreover, the expansion of cereal monocultures and the reduction or abandonment of crop rotations led to a loss of cultivated diversity and a substantial simplification of agricultural systems (Sans et al. 2013; Ficiciyan et al. 2018; Dainese et al. 2019).

To overcome the simplification of agricultural systems and reduce the dependency on external inputs, organic farming promotes field complexity which means increasing agricultural diversity and ecological interactions among the organisms present to the field (IFOAM 2008). Agricultural diversity includes all organisms that coexist and interact in the agricultural ecosystem, comprising planned diversity, which refers to the organisms intentionally incorporated to the crop, and associated diversity, which includes all naturally occurring organisms in the field, from microorganisms to flora and fauna. Within planned diversity, we can differentiate between specific diversity, the association of two different species, for instance, cash crop and cover crop, and genotypic diversity, such as cultivar mixtures (Andow 1991).

In early organic farming research, complex agricultural systems were believed to achieve benefits like pest and weed control or greater yield (Altieri & Rogé 2009; Dainese et al. 2019). These benefits were later termed agroecosystem services (MEA 2005). However, establishing complex systems does not always result in these services, because interactions between associated cultivars or species can sometimes be negative, such as competition for resources (Barbosa et al. 2009; Gaba et al. 2014; Barot et al. 2017).

In this context, functional diversity was defined by Tilman (2001) as those components of diversity that contribute to beneficial interactions, providing resistance, efficient resource use, stability, and resilience to the agricultural system.

Designing functional polycultures (associations of species or cultivars) in cereal crops must consider several key aspects. First, it is important to select species or cultivars adapted to local agronomic, soil, and climatic conditions (Serra-Gironella & Álvaro 2017; Altieri & Rogé 2009). Second, it is essential to associate plants with different functional traits (Barot et al. 2017; Brooker et al. 2021). Each species or cultivar has specific functional traits (morphological, physiological, and phenological characteristics) that influence crop functionality and consequently the provision of agroecosystem services (Lavorel & Garnier 2002; Gaba et al. 2015). For example, tall wheat cultivars (functional trait) are more efficient in light utilization (ecosystem functionality), giving the crop a competitive advantage over weeds (service) (Worthington et al. 2013). Third, promote positive interactions such as facilitation, complementarity, or compensation between associated species or cultivars (Barbosa et al. 2009; Brooker et al. 2021). For instance, associating a legume cover crop to cereal crops is common due to the facilitation interaction. The legume fixes atmospheric nitrogen to the soil, benefiting cereal development (Wezel et al. 2014).

1. The role of wheat polycultures on providing agroecosystem services

Winter wheat (*Triticum aestivum* L.) is a major global crop. In Catalonia, rainfed cereal crops cover 40.92% of cultivated land, with winter wheat and barley as the primary crops (IDESCAT 2022).

Wheat cultivar mixtures have great potential for providing agroecosystem services due to the genetic diversity among cultivars which gives them different functional traits (Barot et al. 2017). Combining these unique traits can optimize resource use, improving crop functionality and agroecosystem service provision (Costanzo & Bàrberi 2014; Tilman et al. 2014). For instance, cereal mixtures offer nutrient use efficiency (Yan et al. 2018), weed regulation (Kiær et al. 2009), resistance to pathogens (Gibson & Nguyen 2021), resistance to abiotic stresses (Costanzo & Bàrberi 2014), and increased grain production (Reiss & Drinkwater 2018). Although monoculture remains dominant, the use of cereal cultivar mixtures has recently increased in Europe (Costanzo & Bàrberi 2014; Wezel et al. 2014; Borg et al. 2018).

2. Aphid control

Aphids are phytophagous insects considered potential pests in cereal crops due to their impact on production. In wheat crops, aphids directly reduce production by sucking nutrients from the host plant and indirectly by acting as vectors for viruses such as barley yellow dwarf virus (BYDV), among others (Dedryver et al. 2010). Additionally, aphids have been widely used as model organisms in plant-herbivore relationship studies (Rodriguez-Saona & Stelinski 2009).

The process of host selection and aphid population development is complex and comprises different phases (Powell et al. 2006). The first phase is the search for the host plant where aphids use olfactory and visual stimuli to locate their host. The odor profile is the set of volatile compounds constantly emitted by a plant, used for plant-to-plant communication. It contains information about the plant's identity and quality. Aphids have an olfactory organ called stylet, which precisely captures these odor signals and uses the information to select the host (Webster 2012).

The second phase is acceptance, where aphids evaluate the host's quality through olfactory, tactile, and gustatory stimuli. After host acceptance, the aphid population develops. The population's growth and aphid size depend on the host's quality (Aqueel & Leather 2011; Nowak & Komor 2010).

Pest control in wheat cultivar mixture is related to two sets of ecological mechanisms: bottom-up and top-down. When aphid population regulation relies on resistant cultivars or species it is known as bottom-up control. Conversely, whether it comes from higher trophic levels, such as predatory arthropods (coccinellids, spiders, etc.) and parasitoid wasps, it is called top-down control mechanism (Barbosa et al. 2009; Gurr et al. 2017).

Bottom-up control mechanism is directly linked to planned diversity. Facilitative relationships between resistant and susceptible cultivars provide associational resistance to the cropping system (Barbosa et al. 2009). This associational resistance can result from positive interactions that induce morphological or physiological changes, increasing the resistance of susceptible cultivars (Barbosa et al. 2009; Ninkovic et al. 2016; Dahlin et al. 2020) or from the visual and olfactory concealing of susceptible cultivars by resistant cultivars, making them harder for aphids to locate (Tahvanainen & Root 1972; Finch & Collier 2000).

To enhance bottom-up mechanisms, it is essential to study the functional traits of wheat cultivars that offer aphid resistance, their complementation, and the influence of plant-plant interactions on these selected traits (Lithourgidis et al. 2011; Gaba et al. 2015; Brooker et al. 2021). Three main functional traits related to aphid population control are the odor profile, tissue nitrogen content and above-ground biomass. While the odor profile of wheat cultivars plays a direct role in the host location and selection phase (Pickett et al. 1992; Webster 2012), the nitrogen content and above-ground biomass are linked to host quality and, consequently, to the growth rate of aphid populations (Aqueel & Leather 2011; Nowak & Komor 2010).The nitrogen content of plant tissue is a limiting factor in the diet of aphids, as its concentration in the phloem is low (~0.1 N) (Taiz and Zeiger 2006). Experimental studies conducted under greenhouse conditions have shown a positive relationship between the size, longevity, and fecundity of aphids and the intensity of nitrogen fertilization (Duffield et al. 1997; Aqueel & Leather 2011; Nowak & Komor 2010).

Top-down control mechanisms rely on the concept that complex cropping systems create suitable conditions to attract beneficial auxiliary fauna such as aphid predators and parasitoids (Koricheva & Hayes 2018). Among other functions, planned diversity can provide food resources for beneficial fauna, such as places to lay eggs, seek refuge, and alternative hosts (Gurr et al. 2017).

3. Weed regulation

One of the principal challenges in organic arable crops is managing weed populations, as the application of herbicides is prohibited, and mechanical control is sometimes insufficient to reduce populations below the competitive threshold. Few studies have analyzed the effect of associating different wheat cultivars on weed control. These studies suggest that combining cultivars that differ in height, above-ground biomass, and tiller number per plant could increase competitive capacity by more efficiently occupying aboveground space, and therefore uptake light resources, and also water and soil nutrients from soil (Worthington et al. 2013; Lazzaro et al. 2018).

4. Crop yield

One of farmers' main concerns when considering the incorporation of planned diversity into their fields is maintaining or increasing crop yield and providing yield stability (Di Bene et al. 2022).

Several studies have indicated that associating modern cereal cultivars with traditional ones provides the polyculture greater resilience to adverse environmental conditions and, consequently, greater yield stability (Reiss and Drinkwater 2018; Ficiciyan et al. 2018; Serra-Gironella 2020). The meta-analytical study by Borg et al. (2018) shows that the production of mixed winter wheat cultivars is between 4% and 6% higher than that of respective monocultures.

Additionally, combining cultivars with different biological traits such as height, aerial biomass, and phenological development can promote complementary interactions that result in better light and soil nutrient utilization, and consequently improving crop production (Worthington et al. 2013; Lazzaro et al. 2018).

Justification

Recently, investigations in organic agriculture have highlighted the importance of diversification, from the farm scale to the landscape scale, as a mechanism to enhance weed regulation, pest control or increased yield (Costanzo & Bàrberi 2014; Hatt & Döring 2023).

Research in cereal polycultures has also revealed that it is necessary to consider the identity and interactions between the combined species and cultivars to ensure crop functionality, as well as the importance of field research to assess the development of polycultures in real farm conditions. In this regard, early studies on the role of wheat cultivar mixtures in providing weed and aphid control, have primarily been conducted through experiments in controlled laboratory or greenhouse conditions. These experiments often fail to consider the influence of environmental or agronomic factors on the performance of polycultures and the effects of cultivar mixtures on associated beneficial biodiversity, such as aphid predators or parasitoid wasps.

Additionally, the trait-based approach is frequently overlooked, which can result in nonfunctional polycultures due to redundancies or negative interactions between the associated cultivars.

Furthermore, studies that do consider functional traits often analyze them in individuals grown under monoculture conditions, neglecting the impact of plant-plant interactions on the target traits (Gaba et al. 2015; Barot et al. 2017; Brooker et al. 2021).

Each of the four chapters that constitute this doctoral thesis adopts a unique approach to addressing aspects of wheat cultivar mixture research on weed regulation, aphid control, and crop yield that have received less attention until now.

The first two chapters report the results of field experiments conducted in the Espai d'Interès Natural (EIN) of Gallecs. Gallecs is a periurban agricultural area of 698.91 ha located 15 km north of Barcelona (Mollet del Vallès). Since 2005, the Agroecological Association of Gallecs has actively promoted a transition towards organic farming, emphasizing the sowing of traditional wheat and legume cultivars (Chamorro et al. 2017). In both experiments, we analyze the effect of wheat polycultures on the delivery of the mentioned agroecosystem services.

The selection of the wheat cultivars and the cover crop studied was carried out through a participatory process with the farmers of the Agroecological Association of Gallecs and local millers, as well as with the technical direction of the Consortium of the EIN Gallecs. The choice of wheat cultivars was based on criteria such as the representativeness in Catalonia, nutritional value, cultural value, and commercial purpose from a stakeholder perspective (Serra-Gironella 2020). From a scientific point of view, morphological, phenological and physiological traits such as susceptibility to aphids and height of the different cultivars were also considered.

The selected cultivars were three soft winter wheat cultivars *Triticum aestivum* L. subsp. *aestivum* (Florence-Aurora, Xeixa, and Montcada) and one durum winter wheat cultivar *Triticum turgidum* L. subsp. *durum* Desf. (Husn.) (Forment). Although technically Forment wheat is a different species, for simplicity, we will refer to it as a cultivar. As a legume cover crop, burclover (*Medicago polymorpha* L.) was sown, chosen for its adaptation to the Mediterranean climate and its prostrate growth habit, making it an excellent choice as a cover crop.

The first study (*chapter 1*) addresses the need to delve deeper into the influence of soil management practices on the delivery of agroecosystem services. This study examines the impact of tillage intensity (conventional tillage using moldboard plough which turns the soil over at a depth of 25 cm versus reduced tillage, employing chisel plough which operates at the same depth but with no soil inversion) and fertilization (incorporation of composted manure versus no manure) on the role of wheat crops on weed regulation, aphid control and yield stability. Weed regulation was estimated by weed biomass, aphid control was analyzed by aphid abundance per plot, the number of aphids per tiller at peak time and parasitism rate and crop yield was evaluated at harvest time. The wheat crops studied included a cultivar mixture of one modern cultivar (Florence-Aurora) and two traditional ones (Forment and Xeixa), as well as their respective monocultures. The study was conducted on a long-term trial over two growing periods between October 2018 and June 2022.

The second study (*chapter 2*) shows the results of a field experiment comparing the effects of different wheat polycultures on aphid control and wheat yield. Aphid control was assessed by measuring aphid abundance per plot, the number of aphids per tiller at peak time, the abundance and richness of foliage and ground dwelling predators per plot and parasitism rate. This experiment also evaluated the role of bottom-up and top-down mechanisms involved in aphid control in wheat polycultures. The studied crops included a cultivar mixture of a modern and a traditional cultivar with similar morphological and phenological traits (Florence-Aurora and Montcada) and a mixture of a modern and a traditional cultivar with different traits (Florence-Aurora and Forment), their respective monocultures and also the incorporation of burclover undersowing in each of the previous five crop treatments. The study was carried out on five commercial fields over two growing periods from October 2019 to June 2022.

The last two chapters focus on assessing the bottom-up mechanisms behind aphid population control observed in the field. For this purpose, we characterized three functional traits, and analyzed the interactions (plant-plant and plant-aphid) at different stages of the host selection and aphid population development process under experimental greenhouse and laboratory conditions. The experimental part of these studies was conducted at the SLU (Swedish University of Agricultural Sciences) between April and June 2022. The wheat treatments studied were the same as in *chapter 2*: Florence-Aurora and Montcada, Florence-Aurora and Forment mixtures, and their respective monocultures.

The third experiment (*chapter 3*) evaluates the effect of plant-plant interaction on aphid acceptance rate and aphid population growth rate through controlled laboratory and greenhouse experiments. Additionally, it characterizes the nitrogen content in wheat tissue and above-ground biomass for plants grown in monocultures and mixtures. Finally, the study examines the effect of wheat tissue nitrogen content on aphid growth rate. Although there is extensive literature on the relationship between nitrogen and aphid populations, most research has focused on the relationship between soil nitrogen fertilization and aphid abundance in cereal monocultures (Duffield et al. 1997; Nowak & Komor 2010; Gash 2012).

The last experiment (*chapter 4*) studies the role of the odor signals emitted by wheat plants during the host location and selection phase of the aphid infestation process. First, we identified the composition and volatiles ratios of the odor profiles from the three monocultures. Simultaneously, the odor profile of the mixtures was characterized to analyze the effect of plant-plant interactions on this trait. Finally, we analyzed aphid’s attraction or repulsion responses to wheat treatment odor signals using the olfactometer method (Glinwood et al. 2009).

Discussion

1. The effect of wheat cultivar mixture on weed and aphid control

One of the principles of organic agriculture is the prohibition of external inputs, such as herbicides, insecticides, etc. This implies the need to seek alternative strategies for weed and pest management. Therefore, it is essential to promote the establishment of wheat cultivars that can compete with weed populations and resist pests. To address this challenge, one of the objectives of this doctoral thesis is to evaluate the effectiveness of various wheat cultivars monocultures and wheat cultivar mixtures in weed and aphid control in field experiments. In this doctoral thesis, the abundance of weed populations was estimated by biomass per unit area (*chapter 1*), and aphid abundance was estimated by the sum of aphids per plot (*chapters 1 and 2*).

Our experiments in a long-term trial and in commercial fields revealed that monoculture of the modern wheat cultivar Florence-Aurora was highly susceptible to aphid and weed infestation. In contrast, monocultures of traditional cultivars, e.g. Forment, Xeixa, and Montcada, were more competitive against weed populations and more resistant to aphids than the monoculture of modern cultivar. Moreover, the mix of the three wheat cultivars Florence-Aurora, Forment, and Xeixa resulted in a reduction of weed biomass under high infestation conditions, as in plots where chisel was employed, and a decrease in aphid abundance compared to Florence-Aurora monoculture. The lower abundance of aphids was also observed in the association of Florence-Aurora with Forment.

The benefits of the Florence-Aurora, Forment and Xeixa mixture in weed control can be attributed to their complementary heights, which result in more efficient aerial space occupation. Combining tall traditional cultivars with shorter ones enhances the crop's overall competitive capacity against weeds (Worthington et al. 2013; Lazzaro et al. 2018) (*chapter 1*). Additionally, complex above-ground structure resulting from wheat cultivar mixture can also aid in pest control. Tall cultivars act as a physical barrier that hinders aphids’ mobility and interferes with the visual location of the shorter modern cultivars, which are often more susceptible to pests (Finch & Collier 2000; Ficiciyan et al. 2018) (*chapters 1-2*).

The functionality of wheat polycultures is conditioned by the identity and interaction between the combined cultivars, as the good neighbor hypothesis suggests (Ninkovic et al. 2002; Dahlin et al. 2018). The combination of two morphologically and physiologically similar cultivars, for instance the association of Florence-Aurora with Montcada, has been less effective in aphid control. In contrast, the combination of cultivars with different and complementary functional traits (height, phenology dates of tillering and flowering, tissue nitrogen content, and odor profile), such as Florence-Aurora with Forment and Florence-Aurora, Forment and Xeixa, showed significantly lower aphid abundance compared to susceptible cultivars monocultures.

The results obtained in this doctoral thesis are notable compared to previous research that did not consider trait-approach when designing experiments. In the study by Lazzaro et al. (2018), associations of three and six wheat cultivars presented similar weed biomass to monocultures. Regarding pest control, early field experiments did not observe an effect of wheat polycultures on aphid population (Koricheva & Hayes 2018; Mansion-Vaquié et al. 2019), while greenhouse studies have evidenced aphid control only when three or more wheat cultivars are associated (Shoffner & Tooker 2013; Grettenberger & Tooker 2016).

1.1 The influence of soil management practices on polycultures performance

A key aspect on this doctoral thesis is to analyze the influence of tillage intensity (conventional tillage employing moldboard plow versus reduced tillage using chisel plow) and fertilization (fertilized vs no fertilized) on aphid and weed abundance, and crop yield. As well as the effect of these practices on the performance of Florence-Aurora, Forment, and Xeixa mixture and its monocultures (*chapter 1*).

Our study demonstrated that conventional tillage decreases weed abundance by 22.14% in 2019 and 19.89% in 2021. This pattern was already noted by Baldivieso-Freitas (2018) during the first four years of the same long-term experiment. Conventional tillage also boosted aphid infestation, possibly due to the reduction in weed species abundance and diversity, which negatively affects the attraction of beneficial auxiliary fauna (Hatt et al. 2018). Conversely, nitrogen fertilization did not influence weed nor aphid abundance.

Additionally, under high intensity tillage (conventional tillage) weed abundance was equivalent across wheat treatments. However, in low intensity tillage (reduced tillage), which lead to higher weed infestation, monocultures of tall traditional cultivars (Forment and Xeixa) and the cultivar mixture enhanced weed regulation. Concerning aphid control, the benefits of the more resistance wheat monocultures and the cultivar mixture were revealed indistinctly of tillage or fertilization conditions.

1.2 The effect of burclover undersowing in wheat monocultures and cultivar mixtures

We also assessed the effect on aphid control of incorporating a burclover cover crop in Florence-Aurora, Montcada and Forment monocultures, as well as Florence-Aurora with Montcada and Florence-Aurora with Forment mixtures. The use of a burclover undersowing decreased aphid population when associated with aphid susceptible wheat monocultures, such as Florence-Aurora and when incorporated in Forment monocultures. Since aphids are specialized phytophagous insects, the incorporation of a non-cereal cover crop may conceal the visual and olfactory signals of wheat (Tahvanainen & Root 1972). Additionally, legume undersowing can physically hinder aphid mobility through cereal plants (Mansion-Vaquié et al. 2017). Contrary to expectations, burclover's effects on pest control were not related to top-down mechanisms, as it did not increase the abundance and richness of beneficial auxiliary fauna such as aerial and ground-dwelling predatory arthropods and parasitoid wasps (Lopes et al. 2016; Hatt et al. 2018).

Wheat polycultures associated with burclover undersowing did not overcome the benefits of a single diversification practice. Both cultivar mixtures and burclover cover enhanced aphid control through bottom-up mechanisms. The lack of complementary functions, for instance combining top-down and bottom-up mechanisms, offered by the associated cultivars and species, resulted in a functionally redundant polyculture (Barbosa et al. 2009; Gaba et al. 2015; Barot et al. 2017).

2. Bottom-up control mechanisms related to wheat cultivar mixture

In field experiments, we compared the number of aphids per tiller in cultivars grown in monocultures and polycultures (mixes of three cultivars: Florence-Aurora, Xeixa, and Forment;

mixes of two cultivars: Florence-Aurora with Montcada and Florence-Aurora with Forment; and the incorporation of a burclover cover crop) to assess the bottom-up mechanisms involved in pest control (*chapters 1-2*). The experiments showed a lower number of aphids per Florence-Aurora tillers when associated with resistant wheat cultivars with complementary traits (Xeixa and Forment or just Forment) and when associated with burclover undersowing (chapters 1-2).

2.1 Identification of functional traits related to aphid resistance on wheat cultivars

The experiments conducted in controlled greenhouse and laboratory environments delved deeper into the bottom-up mechanisms observed in the field. Three functional traits were characterized: the odor profile using gas chromatography (*chapter 4*), the tissue nitrogen content by Dumas's combustion method and the above-ground biomass (*chapter 3*) of the same cultivars studied in the field: Florence-Aurora, Montcada, and Forment, grown in monocultures and in mixtures (Florence-Aurora with Montcada and Florence-Aurora with Forment). These attributes were analyzed because the odor signals play a crucial role in the host location phase (Webster 2012; Ninkovic et al. 2016) and wheat tissue nitrogen and above-ground biomass are related to the nutritional quality of the host and aphid abundance (Aqueel & Leather 2011).

The results revealed that wheat cultivars presented different odor profiles, tissue nitrogen content and above-ground biomass. Specifically, Florence-Aurora and Forment showed different olfactory profiles, and Forment exhibited a lower tissue nitrogen content and biomass than the other two cultivars. This variability in functional traits suggests that cultivars can differ in aphid resistance and indicates that combining cultivars with complementary functions can enhance associative resistance in cultivar mixtures (Barot et al. 2017).

The association of cultivars with different odors, like Florence-Aurora and Forment, modified the mixture odor profile. The odor profiles of Florence-Aurora and Forment monocultures and the mixture had the same volatiles composition but differed in volatiles ratio. Concerning host quality traits, Florence-Aurora plants grown with Forment presented a significant reduction of nitrogen content. These alterations could positively impact the associative resistance of the polyculture (*chapters 3-4*).

The effect of combined cultivar's identity in modifying wheat mixtures attributes was also reported by Dahlin et al. (2020). Our study took a step further, suggesting that morphological or physiological alterations mainly occur when associating cultivars with different morphological, physiological and phenological traits.

2.2 Study of plant-plant and plant-aphid interactions

In parallel with the characterization of wheat cultivars functional traits, plant-plant and plant-aphid interactions were evaluated. For plant-aphid interactions we use a population of *Sitobion avenae* (Fabricius) (*chapters 3 and 4*).

Host location phase

The plant-aphid interaction during the host location phase was assessed using olfactometers (Glinwood et al. 2009). This device is used to study insect behavior in the presence of two olfactory stimuli from two treatments being compared simultaneously. Thus, this study presents relative preferences between treatments instead of absolute results. The olfactometer also allows testing aphid rejection responses by comparing the olfactory stimuli of a treatment against a negative control (clean air) (*chapter 4*).

Aphids were attracted equally by the odor signs of the three cultivars grown in monoculture. Therefore, the higher susceptibility of Florence-Aurora cultivar is not related to the emission of more attractive olfactory signals. Secondly, aphids did not have a clear preference between odor signals from the Florence-Aurora with Montcada mixture and those of the respective monocultures, because these treatments have similar odor profiles. Aphid preferred the odor signals of Florence-Aurora over the odor of the Florence-Aurora with Forment mixture. Moreover, aphids were more attracted to the sum of the odor signals of Florence-Aurora and Forment grown in monocultures over the signals emitted by the mix. Finally, aphids had a rejection response only towards the odor emitted by the Florence-Aurora with Forment mixture. These results indicate that combining cultivars with different odor profiles can enhance associative resistance by emitting unattractive or even repulsive odor signals, negatively interfering with the host location and selection process.

Host acceptance phase

The host acceptance phase was analyzed by counting the number of aphids fixed on a wheat plant after 2 hours of contact. The plants used in this test had been previously exposed for a week to the volatile compounds of Florence-Aurora, Montcada, or Forment.

The results of the experiment showed that aphid acceptance of Forment plants was lower when exposed to Florence-Aurora volatiles (*chapter 3*). These results are consistent with the work by Ninkovic et al. (2002), which indicates that plant interactions through volatiles influence the host selection process. This decrease in acceptance could result from alterations in the emitted volatiles, as evidenced by the odor profile analysis, or changes in the plant's texture or nutritional quality. It is noteworthy to highlight that the response was unidirectional: the acceptance of the Forment cultivar decreases when exposed to Florence-Aurora, but not contrarywise.

Aphid population development phase

The greenhouse experiments and field experiments performed similar results concerning aphid population. Florence-Aurora with Forment mixture reduced aphid abundance compared to Florence-Aurora monoculture. Moreover, plants grown in this association had fewer aphids per individual. The trend observed in controlled conditions, where there were no predatory arthropods or parasitoids, indicates that the associative resistance of the cultivar mixture is derived from bottom-up mechanisms. One possible explanation of the associational resistance could be the lower tissue nitrogen content in Florence-Aurora plants when combined with Forment. Cultivars with lower nitrogen content can support smaller aphid populations, since nitrogen is a limiting nutrient for aphids' diet. However, no strong relationship between nitrogen content and aphid abundance was found (*chapter 3*).

3. Top-down control mechanisms related to wheat cultivar mixture

In field experiments, top-down control mechanisms have been directly analyzed through parasitism rate (*chapters 1 and 2*) and indirectly through the abundance of foliage and ground-dwelling predators (*chapter 2*). Parasitism rates and the abundance of foliage-dwelling predators (ladybugs, spiders, and syrphid larvae) were measured using visual counts along linear transects. In contrast, ground-dwelling predators (spiders, carabids, and staphylinids) were collected using pitfall traps.

Our results indicate that crop diversification in wheat fields had little impact on parasitism, as no significant differences were detected among treatments, or these differences were not consistent across sampling periods. These findings align with the meta-analysis by Koricheva & Hayes (2018), which shows a weak relationship between planned diversity and the abundance and diversity of parasitoids, as well as parasitism rates. Moreover, parasitism rates in cereal crops seem to be more influenced by the presence of flower strips and woody fields margins than by diversification strategies within the field (Dassou & Tixier 2016; Salat-Moltó et al. 2023).

The most abundant foliage-dwelling predators were ladybugs in both larval and adult stages (57.0%), while spiders (58.3%) and staphylinids (32.3%), both generalist predators, were the most abundant ground-dwelling predators. According to previous research, the increased crop aerial complexity provided by wheat cultivar mixtures did not promote the abundance or richness of predator arthropods (Lopes et al. 2016; Mansion-Vaquié et al. 2017). One possible explanation is that cereal mixtures do not offer secondary nutritional resources (e.g., pollen and nectar) or additional shelter that may attract beneficial auxiliary fauna.

4. The effect of wheat polycultures on crop yield

One of the most important factors for farmers when considering polycultures is their impact on crop yield (Di Bene et al. 2022). Therefore, we compared the grain yield (kg/ha) of monocultures and wheat polycultures in the field experiments (*chapters 1 and 2*). Additionally, we analyzed the phenology of various wheat cultivars to confirm that simultaneous harvest is feasible when they are mixed (*chapter 1*).

The phenology analysis demonstrated that Florence-Aurora was an early flowering cultivar, with a booting date three to four weeks before Xeixa's and Forment's. However, the phenological gap during flowering shortened during grain maturation to about ten days, ensuring a synchronized harvest time (Newton et al. 2010).

Previous investigations have shown that modern cereal cultivars have higher grain yields than traditional ones, especially under low weed infestation (Kaut et al. 2008; Lazzaro et al. 2018).

However, our studies show similar yields between the monocultures of modern and traditional cultivars, as well as polycultures.

Exceptionally, the grain yield of the modern Florence-Aurora monoculture was very low in 2021 season due to adverse environmental conditions, with rainfall in the Gallecs area being 80% below the annual average. Meanwhile, the traditional cultivar monocultures and the polycultures presented yields comparable to previous years. These results suggested that polycultures are more stable than modern cultivars monocultures against potential environmental changes, thanks to the resilience offered by traditional cultivars (Reiss & Drinkwater 2018). Thus, mixing modern and traditional cultivars could be a mechanism to mitigate potential yield losses in unfavorable years.

The incorporation of a burclover crop did not affect wheat grain yield. The average height of the burclover, 10 to 50 cm, is lower than the height of the selected cultivars, ranging from 60 cm to 180 cm. Therefore, it is unlikely that the burclover strongly competed for light or nutrient resources when incorporated into wheat crops. Furthermore, it was expected that burclover enhanced wheat yield due to nitrogen fixation capacity of legumes (Stagnari et al. 2017). Unfortunately, the burclover treatment was only assessed during the first sampling season due to poor establishment in the second year. Consequently, there was insufficient time for the fixed nitrogen to become available to the crop (*chapter 2*).

Regarding soil management, fertilization was the most influential factor on grain yield (Tamburini et al. 2016; Baldivieso-Freitas et al. 2018). Wheat grain yield significantly decreased in unfertilized conditions for both modern and traditional cultivar monocultures, as well as the mixture of Florence-Aurora with Xeixa and Forment. This can be explained by cereals' low nitrogen use efficiency, making nitrogen a limiting nutrient for their development (Diacono et al. 2019).

Grain yield significantly decreased in Florence-Aurora monoculture and Florence-Aurora, Xeixa and Forment mixture when using reduced tillage. This could be due to the highest competition because of greater weed infestation (Kaut et al. 2008; Lazzaro et al. 2018) or due to the lower nitrogen related to reduced tillage practices (Mäder & Berner 2012). In contrast, the yield of traditional cultivar monocultures, Xeixa and Forment, was unaffected by tillage intensity, as these taller cultivars are better competitors against weeds even in reduced tillage conditions (*chapter 1*).

Conclusions

The conclusions obtained from the four chapters that conform the doctoral thesis are the following:

1. Tillage intensity and fertilization influence weed and aphid control, and crop yield. Specifically, moldboard ploughing has decreased weed infestation and has increased aphid abundance, while chisel ploughing and non-fertilized conditions have reduced the yield of the modern Florence-Aurora monoculture and the Florence-Aurora, Xeixa, and Forment mixture.

2. Traditional cultivars deliver ecological functions to the crop that can enhance the provision of agroecosystem services. Our studies show that the associations of the modern cultivar Florence-Aurora with Xeixa and Forment and of Florence-Aurora with Forment favor weed population regulation and aphid control due to the taller height and greater aphid resistance of the traditional cultivars Xeixa and Forment.

3. The effect of cultivar mixture on aphid control depends on the identity and interactions of the combined cultivars. This thesis also suggests that associational resistance is more likely when mixing cultivars with different heights, gluten strength, olfactory profile, and tissue nitrogen, as seen in Florence-Aurora and Forment. In contrast, associating cultivars with similar traits, such as Florence-Aurora and Montcada, is less beneficial on aphid control due to functional redundancy.

4. The abundance and richness of predator arthropods in wheat polycultures (cultivar mixtures and burclover cover) were similar to the values found in monocultures. Additionally, no clear relationship was observed between parasitism rates and aphid populations. Therefore, top-down mechanisms have a low influence on aphid control in cereal polycultures, and the reduction in aphid population abundance observed in field and greenhouse experiments is linked to bottom-up control mechanisms.

5. The undersowing of burclover to the Florence-Aurora and Forment mixture does not provide an additional benefit for aphid population regulation compared to the mixture without burclover undersowing, as both practices favor pest control through bottom-up mechanisms. Therefore, it may be necessary to associate species and cultivars with different ecological functions to avoid redundancy and promote functional polycultures.

6. The associational resistance of wheat mixtures begins in the host location and selection phase. Aphids differentiate and select varieties based on the emitted odor. In this regard, the association of cultivars with different olfactory profiles, such as Florence-Aurora and Forment, modifies the odor of the mixture, resulting in odor signals less attractive to aphids when compared to monocultures emitted signals.

7. Florence-Aurora, Forment, and Montcada wheat cultivars differed in tissue nitrogen content, with Forment having the lowest content. Combining cultivars with different nitrogen contents, such as Florence-Aurora and Forment, decreased the tissue nitrogen content of Florence-Aurora. However, in the greenhouse experiment, no clear relationship was observed between tissue nitrogen content and aphid population growth.

8. In general, monocultures and wheat polycultures have shown similar yields. However, it is worth noting that Florence-Aurora monoculture yield varied significantly between years. In contrast, the association of this cultivar with traditional varieties, Xeixa, Forment, and Montcada, stabilized wheat yield across harvests seasons.

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Alterations in the odor profile of plants in cultivar mixtures affect aphid host-location behavior

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The effect of cultivar mixtures on aphid control is attributed to the masking or alteration of host-preferred cultivar odor cues. However, the underlying physiological mechanism remains unclear. This study assessed alterations in the volatile emissions of wheat cultivars grown together (Florence-Aurora and Forment; Florence-Aurora and Montcada) and the consequences for the olfactory preference of aphids. Volatile organic compounds were collected from wheat plants grown in a laboratory under mixed or monoculture conditions and subsequently analyzed. The odor profiles of Florence-Aurora and Montcada were indistinguishable from each other. However, the odors of Florence-Aurora and Forment grown in monocultures differed significantly from those emitted by their mixture. The Florence-Aurora and Forment mixture induced plant physiological responses that affected the emission of single volatile compounds and, consequently, altered volatile organic compound ratios. English grain aphids (*Sitobion avenae*) were less attracted to the odors of Florence-Aurora and Forment when grown as a mixture than the combination of the odors from Florence-Aurora and Forment monocultures. Moreover, aphids preferred clean air over the odor from the Florence-Aurora and Forment mixture but preferred the odor from the Florence-Aurora and Montcada mixture over clean air. This study highlights the beneficial effects of intraspecific plant diversity on aphid control by altering plant odors in response to plant-plant interactions. The emission of less attractive odor cues consequently affects plant-aphid interactions; hence, less attractive odors are likely to impair aphid host-locating behavior. This effect was exclusive to certain cultivar mixtures, which supports the "right neighbor" concept.

KEYWORDS

aphid host location, genotypic diversity, functionality, plant-plant interaction, plant odor cues, volatile organic compounds, wheat

1 Introduction

As sessile organisms, plants are constantly exposed to several threats, such as adverse ambient conditions, resource competition, and herbivore attacks; hence, they have developed highly sophisticated strategies to guarantee their survival. For instance, plant-plant interaction is a key mechanism for enhancing plant fitness in competitive scenarios, such as cultivar mixtures (Murphy and Dudley, 2009; Ninkovic et al., 2016). Such interactions between cultivars can lead to unfavorable (associational susceptibility) or favorable (associational resistance) associations for neighboring plants that influence their susceptibility to herbivorous insects (Barbosa et al., 2009).

The effect of intraspecific plant diversity on aphid control has been attributed to associational resistance. This suggests that combining the right cultivars reduces herbivorous damage to the host-preferred cultivar by inducing competition-related changes that affect herbivore feeding. These may include the production of anti-herbivore defenses, alteration of host plant quality, or interference in the host location of herbivores (Barbosa et al., 2009).

Furthermore, several studies have demonstrated that the benefits of genotypic diversity are cultivar-specific and depend on the interactions between the cultivar mixtures, leading to the "right neighbor" concept (Dahlin et al., 2018; Kheam et al., 2023). For instance, Ninkovic et al. (2002) tested the aphid control effect of barley cultivar mixtures in a field experiment using various pairs of barley cultivars; they demonstrated that only certain combinations decreased aphid acceptance.

Host selection in aphids is an extremely complex process that involves a variety of sensory and behavioral mechanisms (Powell et al., 2006). Previous studies have emphasized the importance of plant odor signals in aphid host identification, location, and acceptance (Pickett et al., 1992; Webster, 2012). Volatile organic compounds (VOCs) are detected via the antennal olfactory sensilla and can be used to locate host plants prior to settling to determine the quality of the phloem composition (Powell et al., 2006). Hence, understanding the role of plant olfactory cues in aphid host location behavior may lead to improved integrated pest management strategies.

Furthermore, it is well-documented that plants respond to neighboring volatiles, causing morphological and physiological modifications (Callaway and Walker, 1997; Ninkovic et al., 2016; Ninkovic et al., 2021). For instance, VOCs from herbivore-damaged plants can trigger defensive responses in neighboring undamaged plants (Glinwood et al., 2009; Midzi et al., 2022). Furthermore, VOCs from undamaged plants can induce physiological shifts in neighboring plants, increasing cultivar resistance to aphids or altering tritrophic interactions (allelobiosis) (Ninkovic et al., 2006; Dahlin et al., 2018; Kheam et al., 2023).

Previous studies have mostly focused on volatile interactions between cultivars and their implications for aphid acceptance (Ninkovic et al., 2002; Dahlin et al., 2018). Less is known about the complete plant-plant interactions when grown together, their physiological response, and their consequences on allelobiosis. Hence, this study sought to investigate these aspects. Additionally, gaining a deeper understanding of the physiological mechanisms underlying associational resistance may serve as a foundation for enhancing "right neighbor" combinations and, by extension, as a design tool for functional agrobiodiversity (Barbosa et al., 2009; Gaba et al., 2015; Borg et al., 2018).

Therefore, this study aimed to assess the influence of overall above- and below-ground wheat cultivar interactions, when grown together to simulate field conditions, on the mixture odor profile and its subsequent effect on aphid host location behavior. Two previously field-tested wheat mixtures with varying aphid control capacities were compared. We hypothesized that (i) genotypic diversity effects on aphid host location would be specific to the cultivars combined, (ii) the coexistence of cultivars in the mixture would induce changes in their volatile profiles, and (iii) aphids would prefer odor cues from monocultures over those from cultivar mixtures.

2 Methodology

2.1 Plants and insect material for the experiments

Three winter wheat cultivars were used in the experiments: the modern cultivar Florence-Aurora (*Triticum aestivum* L. *subsp. Aestivum)* and the traditional cultivars Forment (*Triticum turgidum* L. *subsp. Durum*) and Montcada (*Triticum aestivum* L. *subsp. Aestivum).* Seeds were supplied by farmers from the Gallecs Agroecological Union and technical personnel from the Gallecs Natural Interest Area Consortium in Catalonia, Spain. The three wheat cultivars were either grown as monocultures: Florence-Aurora (FA), Forment (FO), and Montcada (MO), or in two cultivar mixtures: 1:1 Florence-Aurora and Forment (FAFO) and Florence-Aurora and Montcada (FAMO). The selection of cultivars and mixtures was based on the farmers' preferences, with Florence-Aurora being the principal cultivar, owing to its excellent bread-making qualities. Both mixtures have exhibited contrasting aphid control abilities in previous field studies (unpublished data). Florence-Aurora was treated as the principal cultivar because it is the most cultivated cultivar in the Gallecs Agroecological Union. Four wheat plants were planted together in plastic pots (13 × 13 × 23 cm) in potting soil (Hasselfors Garden P soil). The plants grew under controlled conditions in a growth chamber at 18–21°C with a 16/8-h light/dark cycle. Olfactometer experiments and headspace collections were conducted on 1-month-old wheat plants. The English grain aphid [*Sitobion avenae* (Fabricius)] was reared on oats (*Avena sativa* L. cv. Belinda) in multiclonal cultures in separate rearing chambers under identical conditions.

2.2 Aphid olfactory response

Aphid olfactory responses to different wheat odors were examined using a two-way airflow olfactometer, which consisted of two stimulus zones in which the odors were introduced and a central zone separating them. Airflow through the system was set to

180 ml min^{-1} measured with a flow meter at the arm inlets (Ninkovic et al., 2013). Plants used as odor sources were placed inside chamber cages directly connected to the olfactometer arms. An adult wingless *S. avenae* was carefully inserted in the middle of the olfactometer using a fine brush. After acclimatization for 10 min, the position of each aphid was recorded at 3 min intervals for 30 min. Each aphid was used only once and was regarded as a replicate. Olfactometers were cleaned with 70% ethanol between trials. Aphids that did not move after acclimatization were excluded from the analysis. The experiment was conducted in a dark room under artificial light (Osram FQ80W/840 HO Constant Lumilux Cool White (4000 K); Munich, Germany) at 60 µmol m^{-2} s^{-1} above the olfactometer to limit the influence of visual inputs. Further, the experiments were conducted on sunny spring days from 9:00 am to 4:00 pm. The average room temperature was 20°C and relative humidity was 40-50%.

First, we compared the monocultures with each other or their respective cultivar mixtures (FA vs. FO, FA vs. MO, FA vs. FAFO, FO vs. FAFO, FA vs. FAMO, and MO vs. FAMO; Figure 1A). Second, we investigated the olfactory responses of aphids to mixed odors from the monocultures. To mix the odor of plants from two pots, each pot was placed in a separate cage, but both cages were connected by a Y-connector to the same olfactometer inlet, resulting in a mixed odor of the two monocultures (FA + FO and FA + MO). Through this method, the odors of the two separately grown monocultures could be introduced on one side of the olfactometer and tested against clean air. Thus, two plants were used as one odor source. We offered this mixed odor simultaneously with the odors of the corresponding cultivars grown in a mixture (FA + FO vs. FAFO and FA + MO vs. FAMO). To equalize the number of plants (biomass), two pots of the cultivar mixtures were connected to an olfactometer, as described for the monocultures (Figure 1B). For the avoidance test, we compared cultivar mixtures (FAFO and FAMO) and mixed odors from monocultures (FA + FO and FA + MO) with clean air (Figure 1C). Each treatment comparison was replicated with 14–27 aphids. Data were analyzed using Wilcoxon matched-pair tests in R, version 4.1.1 (R Core Team, 2021).

2.3 VOC collection

To avoid volatile interactions between the plants, each pot with wheat plants was grown separately inside clear Perspex chamber cages (10 × 10 × 80 cm) (Ninkovic et al., 2002). Air was allowed to enter the forward chamber through an opening in the cage wall (7 cm in diameter), extracted through a tube attached to a vacuum tank, and vented outside the room using an electric fan. The airflow through the cages was 1.3 L min^{-1}. Volatiles were collected using a push-pull system. The upper part of the pot containing the four wheat plants was placed inside a polyethylene terephthalate oven plastic bag (Toppits®; Melitta, Minden, Germany). A self-packed glass liner containing 50 mg of the molecular absorbent Tenax TA (GLScience, Eindhoven, Netherlands) was inserted into the upper opening of the bag. Ambient charcoal-filtered air was pushed into the bag through a Teflon tube inserted into a small hole in the bottom at a flow rate of 600 mL min^{-1} and pulled out over the absorbent at 400 mL min^{-1}. Volatiles were collected for 24 h. At least 10 replications were performed per treatment.

After the collection of VOCs, the aboveground dry weight was measured by cutting all plants per pot above the soil, drying them for 72 h at 60°C, and weighing them afterward. Volatile samples

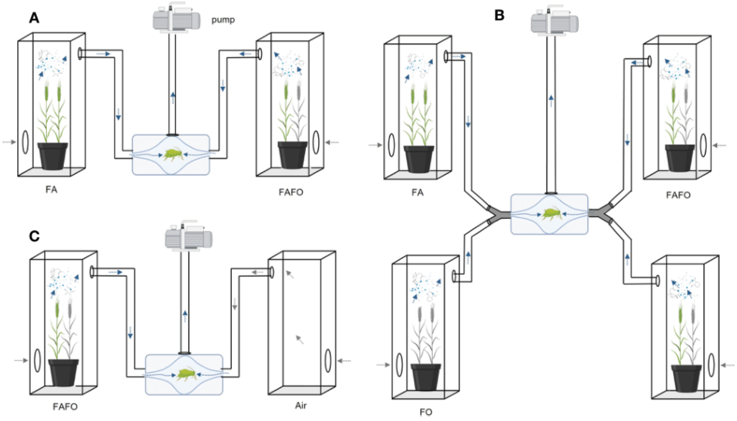

FIGURE 1
Schematic of the experimental design of the olfactometer assays. Pots with four wheat plants were placed in each cage connected to an olfactometer and a suction pump that was used to facilitate airflow from the plants through the olfactometer. An apterous adult aphid was placed in the middle of the olfactometer. **(A)** Representative single-pot comparisons between monocultures and their respective mixtures. **(B)** Representative comparison of two vs two pots. To compare mixed odors of monocultures (one cultivar per pot/cage) and their mixture (two pots/cages of mixtures), two cages were connected using Y-connectors to mix volatile cues before entering each olfactometer arm. **(C)** Schematic of the avoidance test. Aphid movement towards plant odors was compared with clean air. Wheat cultivars: FA, Florence-Aurora; FO, Forment; MO, Montcada.

were analyzed using gas chromatography/mass spectrometry (GC-MS). The sampling tubes were inserted in an Optic 3 Injector (GLScience, Eindhoven, Netherlands), which was heated from 40°C up to 250°C at 30°C/sec to release the volatiles from the absorbent. Helium was used as a carrier gas (Helium 6.0) with a flow of 1.3 mL min ^{1}. The thermal desorbed compounds were separated using an Agilent 7890 N GC system equipped with an HP-1MS capillary column (30 × 0.25 mm inner diameter × 0.25 µm film thickness, 100% dimethylpolysiloxane) and coupled with an Agilent 5975C mass spectrometer (Agilent Technologies, Inc., Santa Clara, CA, USA). The GC temperature program was as follows: Initial oven temperature of 30°C was held for 2 min, increased at a rate of 5°C min^{-1} to 150°C, followed by an increase at a rate of 10°C min^{-1} to the final temperature of 250°C, and then held for 15 min. The temperature of the MS ion source was maintained at 230°C. The quadrupole mass detector was operated in electron impact (EI) mode at 70 eV. The MS gain was set to 10. All data were obtained by collecting the full-scan mass spectra within the range of 40–500 m z^{-} ^{1}. Authentic standards of volatile compounds for identification were measured under the same GC-MS conditions.

2.3.1 Identification and quantification with AMDIS

Volatile compounds from the chromatograms were identified and quantified using the Automated Mass Spectral Deconvolution and Identification System (AMDIS, V. 2.71; National Institute of Standards and Technology, Boulder, CO, USA). Compound identification was based on the comparison of ion fragmentation patterns and retention indices (RIs) of reference standards. Compounds where no standards were available were annotated as unknowns and their ion fragmentation pattern and RIs were used to ensure the comparison of the same compounds between samples, according to the protocol by Gross et al. (2019). After deconvolution, peak areas were integrated for quantification. Identification and deconvolution criteria were applied as follows: match factor, ≥75%; relative retention index deviation, ≤5% from the reference value; match factor penalties level, very strong; maximum penalty, 20; component width, 12; adjacent peak subtraction, 1; resolution, low; sensitivity, very low; and shape requirements, high. Components with a signal-to-noise ratio of <300 were excluded from the analysis.

2.3.2 Statistical analysis

The overall volatile composition of different wheat monocultures and wheat cultivar mixtures as well as the amounts of individual volatiles were compared using multivariate analysis in R (R Core Team, 2021).

To compare the volatile composition, a Bray–Curtis dissimilarity matrix was calculated using the vegdist function from the 'vegan' package (Oksanen et al., 2022). Permutational multivariate analysis of variance (PERMANOVA) of the dissimilarity matrix was calculated using the adonis2 function (N permutations = 10.000). This was followed by pairwise comparisons between wheat monocultures and mixtures using the pairwise.perm.manova function of the 'RVAideMemoire' package (Hervé et al., 2022). P-values were adjusted using the Bonferroni method.

The dissimilarities between the VOC profiles of wheat treatments were visualized with a non-metric multidimensional scaling (NMDS) plot generated with the metaMDS function ('vegan'). We used two dimensions (k = 2) and Wisconsin's double standardization for scaling.

The amount of single volatiles released by wheat plants was analyzed as the peak area/dry weight (g) of aboveground biomass. Kruskal–Wallis rank sum tests were performed to determine the significant differences in the release of single volatiles between wheat treatments. Pairwise comparisons were performed using Dunn's test. P-values were adjusted using the Bonferroni method.

3 Results

3.1 Olfactometer test

To identify the effects of the cultivar mixtures on the olfactory response of aphids, olfactometer experiments were conducted with apterous aphids. When the odors of single wheat cultivars grown as monocultures were offered simultaneously, aphids did not show any preference for one of the cultivars (FA vs. FO: Wilcoxon test: Z = 0.67, P = 0.5, n = 17; FA vs. MO: Z = 1.11, P = 0.26, n = 15). Regarding cultivar mixtures, aphids were more attracted to the odor of FA monocultures than to those of FAFO mixtures when offered simultaneously (Z = 2.78, P < 0.01, n = 25) (Figure 2). No preference was observed for the FO monoculture odors over those from the FAFO mixture (Z = 0.88, P = 0.38, n = 27) (Figure 2).

We compared the mixed odors from monocultures (FA + FO and FA + MO) against odors from the cultivar mixture (FAFO and FAMO) to evaluate whether the decrease in attraction for the FAFO mixture odors was due to changes in VOC emission when Florence-Aurora and Forment cultivars grew together or as a mixture of odors from FA and FO. Aphids preferred the odor cues from FA + FO over those from FAFO (Z = 2.72, P < 0.01, n = 23) (Figure 2). In contrast, the FAMO mixture did not affect aphid behavioral responses (Figure 2). In the avoidance test, FAFO was the only treatment that elicited aphid avoidance (Z = 2.63, P < 0.01, n = 20). As expected, in the remaining treatments, aphids were significantly more attracted to plant odor cues than to clean air: FA + FO (FA + FO vs. air: Z = 2.43, P = 0.05, n = 19), FA + MO (FA + MO vs. air: Z = 2.09, P = 0.05, n = 20), and FAMO (FAMO vs. air: Z = 2.31, P = 0.05, n = 20) (Figure 3).

3.2 VOCs profile

To investigate the impact of intraspecific interactions between wheat cultivars on the volatile emission, the headspace of wheat plants of three cultivars grown as monocultures or in a two-way mixture was sampled and analyzed via TD-GC-MS.

We analyzed the composition of 88 detected compounds emitted by wheat plants. The volatile compositions differed

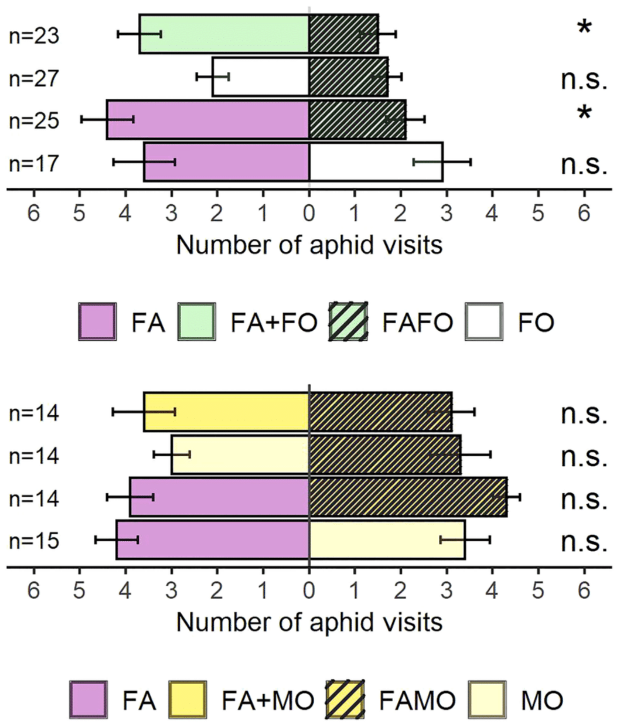

FIGURE 2
Number of aphid visits to wheat plant odor cues. Comparisons related to FAFO mixture: FA + FO (odor mixture of Florence-Aurora and Forment cultivars when grown in monoculture) vs. FAFO (Florence-Aurora and Forment mixture) (n = 23), FO (Forment monoculture) vs. FAFO (n = 27), FA (Florence-Aurora monoculture) vs. FAFO (n = 25), and FA vs. FO (n = 17). Comparisons related to FAMO mixture: FA + MO (odor mixture of Florence-Aurora and Montcada when grown in monoculture) vs. FAMO (Florence-Aurora and Montcada mixture) (n = 14), MO (Montcada monoculture) vs. FAMO (n = 14), FA vs. FAMO (n = 14), and FA vs. MO (n = 15). Error bars indicate the standard error of the mean. Asterisks indicate significant differences according to the Wilcoxon signed-rank test (P < 0.05).

significantly between cultivars in monocultures and mixtures (PERMANOVA, df = 4, R^2 = 76.13, N = 10.000, P < 0.001). Pairwise comparisons showed significant differences in odor profiles of Florence-Aurora and Forment in monoculture (P < 0.01) as well as compared to the FAFO mixture (Figure 4). The odor compositions of the FAMO mixture were not distinguishable from the odor profiles of FA and MO monocultures (Figure 4).

Overall, cultivar mixtures released higher amounts of VOCs than the three monocultures (Figure 5). Particularly, FAFO emitted significantly higher amounts of 33 specific compounds when compared to FA (37.5%) and of 40 compounds when compared to FO (45.4%), including β-caryophyllene, β-ocimene, limonene, 1-octen-3-ol, nonanal, octanal, benzaldehyde, acetophenone among other unknown compounds (Figure 5). VOC emission from the FAMO mixture was similar to that from the FA and MO monocultures, only releasing a significantly higher amount of eight (9.09%) and two (2.27%) compounds, respectively.

4 Discussion

4.1 Aphid olfactory response to cultivar mixtures

The purpose of this study was to assess whether combining wheat cultivars can modify the volatile mixture profile and, consequently, the aphid host-location response. Aphids are extremely sensitive to slight changes in their hosts' odor cues, which they utilize as host-finding signals (Webster, 2012). Therefore, the alterations in mixture odor cues caused by plant-plant interactions can influence the aphid host-locating response and its attractiveness to particular wheat combinations, as demonstrated by our mixture of Florence-Aurora and Forment in olfactometer experiments with *S. avenae*. Moreover, the volatiles emitted by Florence-Aurora with the formation mixture led to avoidance by *S. avenae*. Our results indicated that the Florence-

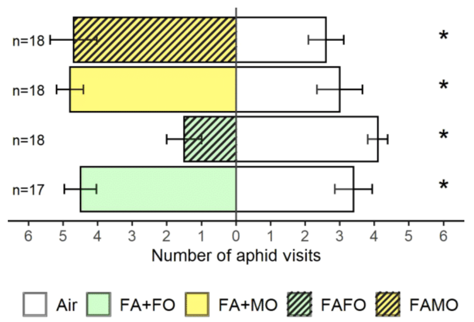

FIGURE 3
Number of aphid visits to wheat plant odor cues and clean air. Wheat treatments: FA + FO (odor mixture of Florence-Aurora and Forment when grown in monoculture) (n = 17), FA + MO (odor mixture of Florence-Aurora and Montcada when grown in monoculture) (n = 18), FAFO (Florence-Aurora and Forment mixture) (n = 18) and FAMO (Florence-Aurora and Montcada mixture) (n = 18). Error bars indicate the standard error of the mean. Asterisks indicate significant differences according to the Wilcoxon signed-rank test (P < 0.05).

Aurora and Forment mixture prevented aphid host localization by releasing non-attractive olfactory signals, thereby reducing aphid acceptance and further aphid infestation. Studies have demonstrated the importance of host-specific volatile compounds and their ratios in the overall composition of aphid host-location behavior (Webster, 2012). For instance, regarding the volatile ratio in olfactometer tests reproducing hop (*Humulus lupulus*) leaf volatile cues, Campbell et al. (1993) found that aphids responded positively to the odor of (E)-2-hexenal and β-caryophyllene in an approximate ratio of 39:1 by weight. However, when the ratio was adjusted to 1:1, no response was observed. Therefore, a shift in the odor cues of Florence-Aurora and Forment cultivars when grown together may influence plant-pest interactions by decreasing crop odor attractiveness to aphids. In contrast, mixing Florence-Aurora and Montcada did not affect the aphids' host-locating behavior compared to the monocultures or the odor mixture of the two cultivar monocultures, which is consistent with the absence of volatile profile alterations shown in the mixture.

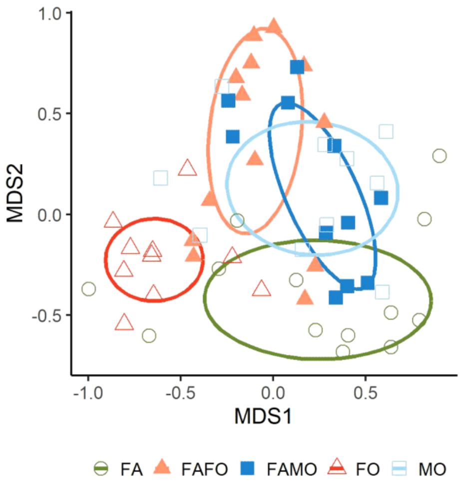

FIGURE 4
Non-metric multidimensional scaling (NMDS) plot illustrating the Bray-Curtis dissimilarities of the proportional volatiles compositions of wheat cultivars Florence-Aurora (FA) (n = 13), Forment (FO) (n = 10) and Montcada (MO) (n = 10) grown in monocultures and in mixtures: Florence-Aurora with Forment (FAFO) (n = 13) and Florence-Aurora with Montcada (FAMO) (n = 11).

4.2 Volatile emission of cultivar mixture

In this study, we assessed the interactions of undamaged wheat cultivars Florence-Aurora with Forment and Florence-Aurora with Montcada when grown together. The results showed that only certain cultivar combinations induced physiological responses to the volatiles emitted when grown together, suggesting the specificity of the effect of genotypic diversity on aphid control. Corroborating with previous experiments, headspace analysis revealed a higher amount of single volatile compounds released from the wheat cultivar mixture than from the monocultures (Shoffner and Tooker, 2013).

Airborne volatiles are crucial signals for inter- and intraspecific plant-plant interactions. Plants constantly emit VOCs and, in return, are constantly exposed to VOCs from damaged and undamaged neighboring plants (Callaway, 2002). These odor cues from emitter plants can affect complex biochemical pathways in the receiver plants (Midzi et al., 2022). Although most studies have focused on the induced defense response in plants receiving VOCs from herbivore-attacked plants (Hu et al., 2019; Midzi et al., 2022), previous studies have demonstrated that VOCs from undamaged plants also trigger morphological and physiological responses in receiver plants (Ninkovic et al., 2016; Kheam et al., 2023). For example, plants of the barley cultivar Kara (*Hordeum vulgare*) allocate more biomass to their roots after exposure to VOCs from cv. Alva compared to the unexposed plants or cv. Kara plants previously exposed to VOCs of another Kara plant (Ninkovic et al., 2003).

We observed physiological responses in the form of altered release of VOCs by the Florence-Aurora and Forment cultivars when mixed. Furthermore, the analysis of specific compound amounts demonstrated that Florence-Aurora and Forment interactions, when grown together, altered the emitted amount of certain compounds, shifting the volatile ratio, which plays an important role in aphid host location (Webster, 2012). Regarding specific volatile chemicals, Visser and Fu-shun (1995) demonstrated that *S. avenae* was attracted to 2-hexanal, benzaldehyde, and linalool, but vaguely responded to 1-octen-3-ol, β-caryophyllene, and limonene odor signals. Moreover, 2-hexanal, linalool, octanal, nonanal, and caryophyllene are assumed to be strong cereal aphid

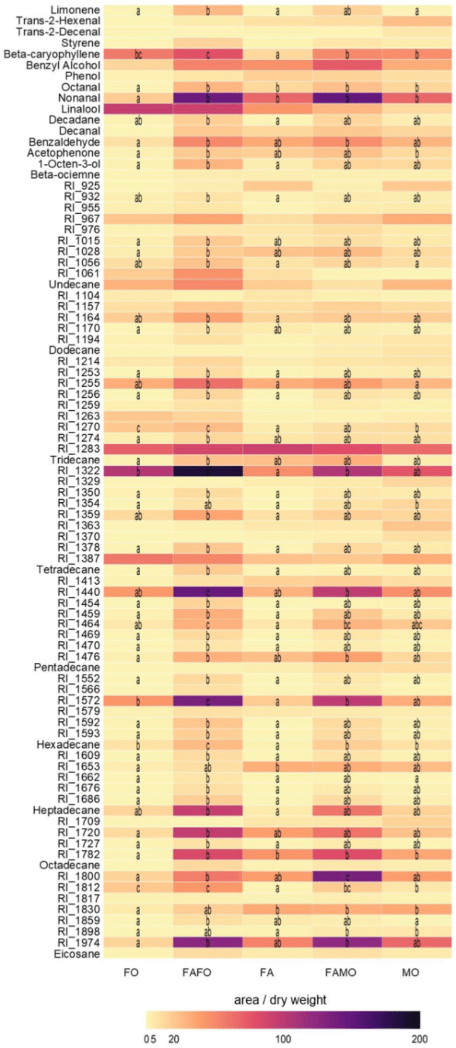

FIGURE 5
Composition of the volatile chemicals obtained by GC/SM of wheat treatments: Florence-Aurora (FA) (n = 13), Forment (FO) (n = 10), Montcada (MO) (n = 10), Florence-Aurora and Forment mixture (FAFO) (n = 13), and Florence-Aurora and Montcada mixture (FAMO) (n = 11). Dark blue indicates a high relative abundance of a respective volatile compound, and light yellow a low abundance. Numbers are mean values of compound abundance (compound peak area/dry weight (g)). Letters indicate significant differences according to Dunn´s pairwise test ($P < 0.05$).

attractants *S. avenae* and *Rhopalosiphum padi* (Linnaeus) (Pickett et al., 1997; Quiroz and Niemeyer, 1998; Birkett et al., 2010). In our study, the abundance of these volatile chemicals was greatest in the Florence-Aurora and Forment mixture, whose odor cues were surprisingly less attractive than those of the monocultures. This suggests that the plant volatile ratio may play a more important role in modifying aphid behavior than the abundance of specific volatiles (Bruce and Pickett, 2011).

Further, the analysis of the odor profile of single cultivars confirmed that the Florence-Aurora cultivar had a volatile profile similar to that of the Montcada cultivar but significantly different from the Forment profile. The interaction between the Florence-Aurora and Montcada cultivars did not affect the mixed odor profile. In line with previous volatile barley experiments, our findings support the hypothesis that greater differences between cultivars' odor profiles might induce greater physiological responses (Dahlin et al., 2018).

In conclusion, our study supports the “right neighbor” hypothesis by demonstrating an intraspecific interaction effect on the odor profile of mixtures, exclusive to certain cultivar mixtures, and might explain the dependence of genotypic diversity on aphid control recorded in numerous field studies (Ninkovic et al., 2002; Dahlin et al., 2018). Our results suggest that the similarity/dissimilarity of VOC emissions is important for plant-plant interactions between the combined cultivars. In the present study, wheat cultivars with distinct profiles affected each other, resulting in an odor that was less attractive to *S. avenae*. Future studies should address whether plants with generally dissimilar odor profiles are more likely to have an impact on each other, and thereby possibly promote associational resistance and enhance aphid control.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

VN, JG, FS, LC-L, and AT-F conceived and designed the research. JG, AT-F, and AE conducted the experiments. JG and AT-F statistically analyzed the data. AT-F wrote the first draft of the manuscript. VN and JG reviewed, commented, and polished the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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