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Disturbance and competition drive diversity effects in cabbage–aphid–onion systems with intra-specific genetic variation

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Abstract

Decreased reliance on pesticides can be achieved through a clever use of eco-evolutionary knowledge via intercropping economically valuable crops with companion plants that can hamper pest outbreaks. We created a greenhouse multi-layered microcosm system to test two potato peach aphid clones, performing alone or in competition, on mixes of genetically variable cultivars of cabbage, with and without onion. The onion acted as a nuisance/disturbance for the pest, which was generally for the benefit of the cabbage albeit both plants sharing space and nutrients. The onion effect was context-specific and differed by aphid genotype. Onion variable nuisance negatively affected the numbers of one aphid genotype (green) across all contexts, while the other genotype (pink) numbers were decreased in two contexts only. However, the green performed better than the pink on all cases of cabbage di-mixes despite its numbers being capped when the onion was present. Further, there was also a general aphid propensity to wander off the plant along with a differential production of winged morphs to escape the onion-affected environments. Moreover, through a comparative increase in dry mass, which was subject to onion and aphid effects, a diversity effect was found where the cabbages of fully genetically variable microcosms sustained similar final dry mass compared with non-infested microcosms. Our findings provide fresh insights into the use of multi-layered contextual designs that not only allow disentangling the relative effects of genetic variation and modes of interaction, but also help integrate their benefits into pest management in view of companion planting.

Keywords: genetic variability, competition, intercropping, disturbance

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Introduction

Pests remain the first cause of yield loss in agricultural settings (Cerda *et al.*, 2017), with arthropods destroying up to 25% of the world's crop production annually (Sallam, 2013; Culliney, 2014). Aphids, in particular, play a significant role in the loss of yield of economically valuable plant species such as legumes and cabbages (Wang *et al.*, 1996; Patel *et al.*, 2004; Razaq *et al.*, 2011). The use of pesticides has been the primary reply to prevent yield loss due to insect infestation (Hooks *et al.*, 2007; Aktar *et al.*, 2009), but it is becoming clearer that the intensive use of chemicals in agricultural settings has health and environmental costs (Wilson & Tisdell, 2001; Wu *et al.*, 2016). Researchers now look for more natural and environmentally friendly, yet efficient, ways to decrease herbivory via integrated pest management techniques (Wilson & Tisdell, 2001; Stenberg, 2017; Khudr *et al.*, 2017*a*). Other agricultural techniques can play with the ecological context in order to

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decrease the negative impact of phloem-feeding pests; among them, sowing crops with natural repellents as companion plants in polyculture settings (Smith & McSorely, 2000; Machado, 2009; Stenberg, 2017).

Companion planting, as a form of intercropping, is a widespread agricultural technique of polyculture consisting of planting more than one species together in the field: a crop plant (primary) and an insect-repellent one (secondary). Although this technique is ancient (Barbera & Cullotta, 2016), its diverse benefits are being re-discovered today (Knörzer *et al*., 2009) as intercropping promises a greener and more sustainable modernized agriculture (Zhang & Li, 2003). For example, it has been shown to reduce N input requirements (Hauggaard-Nielsen, 2001), lead to higher yields (Li *et al*., 2001) and may, as well, decrease the need of pesticide use (Mushagalusa *et al*., 2008). Unfortunately, the main reason intercropping has declined in developed countries is that machineries function more effectively with single-variety arable farming (Machado, 2009).

Research focusing on plant diversity has demonstrated that a worldwide trend of positive diversity effect on productivity exists (Loreau, 1998; Loreau *et al*., 2001; Hooper *et al*., 2005). One of the mechanisms proposed to explain such an increase in productivity is a better protection owing to increased plant variability against specialist herbivores, but this increase in productivity may originate from other mechanisms, as well, such as plant changes in productivity and dominance shifts (Gough & Grace, 1998, but see Kotowska *et al*., 2010). In particular, onion *Allium cepa*, planted as a companion to other cash-crop species, has been shown to effectively repel aphids, which are plant sap-feeding virus transmitters (Hooks *et al*., 2007; Van Emden & Harrington, 2007) because it produces a range of repulsive substances, e.g., sulphuric compounds (Barbosa & Letourneau, 1988; Dancewicz & Gabryś, 2008) that may interfere with aphid reproduction mode and population dynamics (Hooks *et al*., 2007; Vucetic *et al*., 2014). It is common knowledge that onion sap, therefore, does not make part of the diet of the vast majority of aphids, including serious generalists such as potato peach aphid *Myzus persicae* (Sulzer) (Hori & Harada, 1995; Blackman & Eastop, 2000).

The presence of *Allium* sp. on a fine-scale in genetically variable mixed cultures of cash crops such as *Brassica oleracea* can disturb aphid ability to locate, allocate and populate the available resource due to ambiguous or confusing plant-cue mixing (Altieri & Nicholls, 2004; Baluška & Ninkovic, 2010; Ben-Ari & Inbar, 2014). The effects of onion on aphid infestation has been shown by Pahla *et al*. (2014), who recommended intercropping *Brassica napus* with *Allium sativum* and *A. cepa* to control exponential reproduction of cabbage aphid *Brevicoryne brassicae*. Also, Zhou *et al*. (2013) emphasized the utility of garlic *A. sativum* as a pesticide alternative in polyculture for suppressing grain aphid *Sitobion avenae* population. Moreover, on a fine scale, Baidoo *et al*. (2012) demonstrated that onion–cabbage cultures suffered from less intensity of infestation by cabbage aphid *B. brassicae* than homogeneous cultures. Due to communications between plant species and their phloem-feeders, increased plant diversity can have perceptible effects on aphid population dynamics and decision making (Baluška & Ninkovic, 2010). Indeed, companion plants, such as onion, can incorporate the effects of inter-specific genetic variation into the framework of intra-specific genetic variation effects (Tooker & Frank, 2012) in the plot and can, therefore, act as a bridge between agricultural practices and eco-evolutionary research. Nevertheless, despite the increased interest in integrating more intra- and inter-specific genetic variability into the biocontrol of pests (Stenberg, 2017), research on the compound effects of insect-repellent companion planting and intraspecific genetic variation in cash crops and their pests is still meagre.

Aphids have evolved to detect suitable host plants. Their selection is influenced by many factors such as quality (Underwood, 2009), palatability (Powell *et al*., 2006), phenotypic variability (Karban *et al*., 1997), habitat variance (Underwood, 2009) and plant genetic variation (Dungey *et al*., 2000; Whitham *et al*., 2003; Baluška & Ninkovic, 2010). *M. persicae* aphid mothers rely heavily on the gustatory and olfactory cues perceived from their surroundings to determine the suitability of a potential plant as a prospective host for their future colony (Powell *et al*., 2006). Under hostile (unfavourable) conditions, aphids are able to condition alatae; winged morphs produced in one generation and able to escape their adverse environment (Dixon, 1985).

Because the eco-evolutionary arms-race between phloem-feeding insects and their host plants is a complex ongoing phenomenon (Fussmann *et al*., 2007; Hersch-Green *et al*., 2011; Moya-Laraño *et al*., 2014), it is essential to develop a thorough understanding of several layers of genetic and environmental effects that underlie and add to the complex nature of the plant–aphid ongoing reciprocal actions and countermeasures (Mello & Silva-Filho, 2002). Not only host plants and aphids affect each other, but also other environmental inter-players (e.g., companion plants/intercrops) can alter the interactions between, and the responses of, plants and aphids in terms of performance and behaviour (Kanvil *et al*., 2014). For example, in a previous paper (Khudr *et al*., 2017*b*), we have shown that host genetic variability and identities of the competing aphid genotypes, and their interactions, could lead to very different performance outcomes and that would vary by the context of interaction.

Pest competition has been attracting increased research interest (e.g., Petersen & Sandström, 2001; Smith *et al*., 2008). An in-depth understanding, however, is yet to be attained on the impact of intraspecific competition in phloem-feeding insects on host plant communities with intra-specific genetic variation (Tooker & Frank, 2012) under pest disturbance by insect-repellent companion plants. Also, to date, little is known on the effect of the above on crop fitness (vigour, hereafter), especially with the evidence suggesting that polyculture on its own may not be sufficient to control pests of the crop (Smith & McSorely, 2000).

As such, to better understand the interplay between the various environmental, genetic and ecological components that affect the outcome of aphid infestation in polyculture settings, it is necessary to design and conduct experiments that adequately simulate this multi-layered system. In particular, we need to be able to capture the effects of competition between different aphid genotypes on their performance and behaviour within the embedded environment that includes, or not, a repellent companion plant that accompanies genetically variable and vulnerable host plants. We also need to be able to assess whether the advantages of growing with a plant that naturally repels the phloem-feeder can outweigh the potential negative effect arising from plant competition for shared resources.

In the current study, our primary goal was to assess how performance and phenotypic plasticity of two con-specifics

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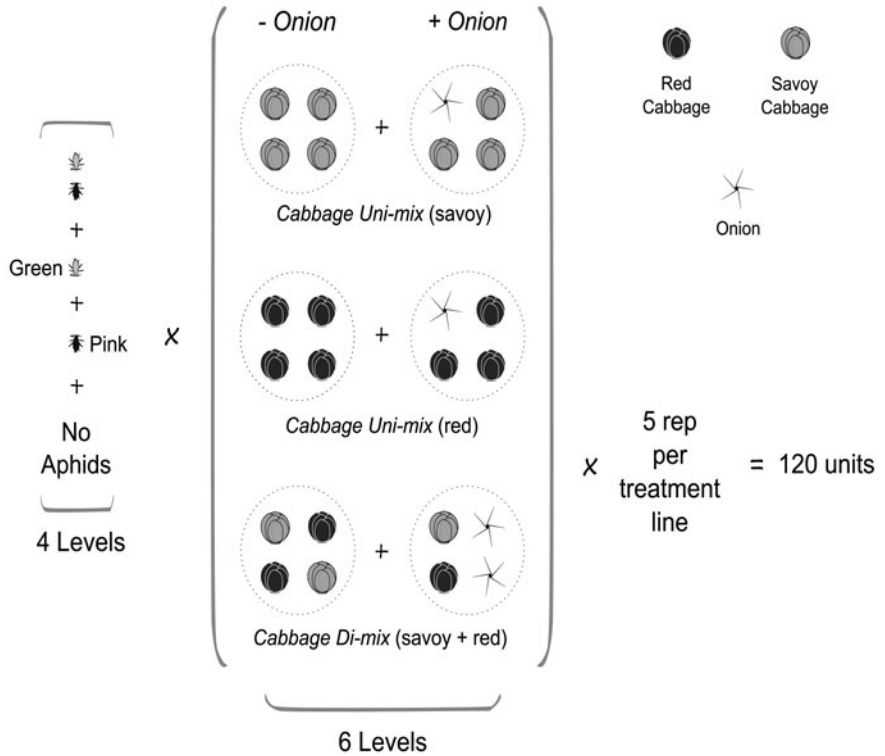


Fig. 1. Experimental design. An illustration of the experiment comprising cabbage genetic variability, aphid clones, aphid competition and the presence of onion. Each of the two conspecifics of potato peach aphid (Green and Pink clones) performed alone or in interaction with its counterpart on distinct environments. Uni-mixes were composed of savoy cabbage or red cabbage, with or without onion presence as an insect repellent, replacing one cabbage plant in each of the uni-mix cultivations. For the cabbage di-mix (savoy and red together) two onion plants were employed instead of two cabbages (one seedling savoy and one seedling red cabbage). In each microcosm there were always four plants. All seedlings were grown at opposing points close to the edges of the pot.

of *M. persicae* could be modulated by their mode of interaction (alone or competing), the genetic variability of the host plant mix on which they perform, and the presence of onion as a nuisance factor by the way of repellence. The host plants were two cultivars of cabbage, an important member of the mustard family. Our second goal was to quantify plant response (vigour) in terms of dry mass under the effects of plant and aphid genetic variability, and the nuisance effect of onion on aphids.

In our approach, the intra-specific genetic variation effects are comprised by two clones on the side of the aphid, and two cultivars on the side of the host plant. We specifically raise the following questions: (1) with and without onion nuisance, how does intra-specific genetic variation of aphid and their host plant affect aphids' performance, behaviour and polyphenism? And (2) what is the plant's response to the multi-layered environment wherein it is growing?

Materials and methods

To test the importance of ecological context and the nuisance/disturbance by onion on the performance and behaviour of aphids and the effects of which on the host plant, we have created a specific multi-layered environmental set-up. We observed the performance and behaviour of two genotypes of potato peach aphid with and without intra-specific competition in the following contexts: on two different cultivars of cabbage (uni-mixes), or a mix of both (di-mix), in the absence and presence of a natural aphid repellent (onion). We have also adopted a plant's perspective to investigate host plant (cabbage) response subject to cabbage genetic variability, aphid genotype and aphid interaction mode (alone or competing), and the onion impact on the ecological context; we present a conceptual schematic diagram in fig. 1.

The organisms

We selected two clones of potato peach aphid *M. persicae* (Sulzer) to infest the plant mixes. One Pink clone was provided by Bayer© (Monheim, Germany) from a lineage that originated in Japan and was sampled therein from *Nicotiana tabacum* (L.). While the Green clone descended from a laboratory-maintained lineage that was originally collected in Germany by the Julius Kühn Institute (JKI), Berlin, Germany. As such, these two aphid conspecifics have different origin and life history and they reflect a portion of the intra-specific genetic variation and colour polymorphism of *M. persicae* in the field. For simplicity, we refer to these different clones, hereafter, as Green and Pink. The Green is usually more virulent than the Pink (pers. observ.) All aphids were raised for few months on Kale *B. oleracea* convar. acephala var. sabellica (L.) before the commencement of the experiment as a way to minimize any maternal preconditioning effects. The seeds of one cultivar (Braunschweiger Dunkelblutrote) of onion *A. cepa* (L.), and

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two cultivars of *B. oleracea* (L.), red cabbage (*B. oleracea* var capitata, cultivar Cabeza negra 2) and savoy cabbage (*B. oleracea* var sabauda, cultivar Vertus 2), were purchased from a local supplier (Sperli©), Germany. For simplicity we refer to the plants, hereafter, as onion, red cabbage and savoy cabbage. The onion line used in this study is a recognized resistant variety. Together with the two cabbage lines, these are the products of intensive selective breeding and they are common cultivars because they show uniformity, stability, productivity, cold tolerance and resistance to pests (Fang *et al*., 2005; Cartea *et al*., 2010; CABI Plantwise Knowledge Bank, 2019). However, the cabbages have intrinsic differences that may stem from genetic differences (Verkerk *et al*., 2010). For instance, pigments (savoy cabbage lacks anthocyanins, which is abundant in red cabbage) (Wiczkowski *et al*., 2013). Anthocyanins have been reported to confer protection on plants against pests (Cartea *et al*., 2010; Suszkiw, 2011), perhaps more due to ingestion rather than mere leaf colouration (Green *et al*., 2015). Furthermore, differential age-dependent and line-dependent content of amino acids, sulphur and defensive metabolites (e.g., glucosinolates) may exist between conspecifics (Castro *et al*., 2004; Bellostas *et al*., 2007) along with distinct leaf morphology (blistered in savoy cabbage but smooth in red cabbage). With a simpler leaf surface/texture, red cabbage phloem is more reachable by aphid stylets than that of savoy; leaf ontogeny also can pose a degree of ecological challenge to aphids as older leaves are less penetrable than younger ones hence the latter are more preferable to colonize (Vickers, 2012; Cao *et al*., 2018). Comprehensive information, however, on cultivar by genotype adverse effects of anthocyanins and glucosinolates on aphid performance is thus far lacking (*nota bene*).

The set-up

The experiment took place in a greenhouse of the Freie Universität Berlin. The temperature in the greenhouse was constant around 22–24°C and the photoperiod was set to long days (16:8 h cycles).

Four plants were sown together in stream-sterilized soil mixed with perlite (3:1 ratio) using 6″ (~15 cm) pots. Three weeks after germination, they were enclosed in plastic transparent cylinders (40 cm height) to prevent aphids from escaping. Two fine-meshed windows (15 × 10 cm^2) were crafted for ventilation. The four plants were sown either alone or in a mix, with and without the presence of onion, making six possible plant assemblages (fig. 1). The plants were sown at opposing points close to the edges of the pot. The microcosms were placed in rows. Watering occurred when needed by filling the individual trays beneath each microcosm with water.

Before enclosing the microcosm including four 3-week old plants, we infested each microcosm with eight second nymph aphid instars. The aphids were deposited at the centre of the microcosm and hence free to choose the plant/s to infest. We deposited either eight Green or Pink aphid nymphs per microcosm or four of each in the case of intra-specific competition (fig. 1). Finally, we had a control treatment with no aphid infestation for a total of six possible aphid assemblages (fig. 1).

We replicated each plant × aphid assemblage five times and, therefore, the experiment had 120 experimental units (microcosms). The experiment lasted for 24 days after aphid introduction; that was to enable the duration of the experiment to span the development of aphid instars into maturity and subsequent production of one generation of offspring. It was necessary to examine the effects of maternal effects of parthenogenetic aphids in preconditioning their progeny across contexts that varied in food availability and hostility (nuisance/repellence as a form of disturbance).

The measures and their analyses

All analyses were done in R, version R-3.4.0 (R Core Team, 2017). We used parsimonious regression models with the family distribution adapted for the data at hand. Only the significant results are shown in the Results section, full model results are available in Supplementary Materials.

Aphid traits

At the end of the experiment, we estimated aphid performance by counting aphid total numbers per microcosm. Here, we integrated the shoot-to-root ratio as a covariate indicating plant dry mass (aphids feed on the shoot sap) into the analysis of aphid performance (see below). Our motivation for taking such an extra measure is the assumption that plants with a lower shoot-to-root ratio will affect aphid performance (Windle & Franz, 1979). Shoot-to-root ratios can also be helpful in determining the indirect effect of above-below ground plant–plant interaction on both plants and aphids. There could be either facilitation or competition between the two cabbage hosts and the repulsive onion (Zhang *et al*., 2012; Geem *et al*., 2013). The explanatory variables were: (1) plant dry mass (shoot-to-root ratio) as a covariate indicating relative plant differential fitness and food availability for aphids. (2) Onion (0 = [0 means four host plants (cabbage) per microcosm and onion plants are absent] that is the baseline of this effect [0.25 means three host plants (cabbage) per microcosm with one onion only present] and [0.5 means two host plants (cabbage) per microcosm with two onion plants only present]). This effect reflects the proportional numerical host plant (cabbage) to repellent plant differences per microcosm. (3) Cabbage genetic variability (categorically: uni-mix1 [savoy cabbage] that is the baseline of this effect, uni-mix2 [red cabbage], di-mix [savoy-red cabbages]. (4) Aphid genotype (Pink [baseline], Green). (5) Aphid interaction mode (alone [baseline], competing). (6) All possible interactions between the main effects (2–5).

For *aphid performance*, we ran a generalized linear mixed effect model, with the *lme4* R package, 'bobyqa' optimization (Bates *et al*., 2015) and *car* package (Fox & Weisberg, 2011). A Poisson family was used to account for the non-normality and skewness of data; the microcosm was considered a random factor. The model was followed by a visualized post-hoc Tukey's honestly significant difference (HSD) test to further highlight pairwise differences of aphid performance across contexts.

As for *aphid behaviour*, first, we recorded and calculated the proportions of aphids off-plants relative to the whole aphid population in the microcosm at the end of the experiment. This was investigated using a generalized linear model (GLM) with gamma family due to high positive skewness with the *multcomp* package (Hothorn *et al*., 2008). The explanatory variables mentioned above were used. Additionally, we surveyed aphid aggregation on plants (stems vs. leaves, results are given in Supplementary Materials, table S6).

We also counted the number of *winged-morph aphids* (alatae) signifying aphid polyphenism (dispersive phenotypes). For the winged aphids, we did not separate the Pink from the Green alatae because they are impossible to distinguish

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with the naked eye. This trait was tested using a separate GLM model with a quasi-Poisson family. We used the same explanatory variables as above except for the indistinguishable aphid genotype. As such, the explanatory variables were (1–3, 5, and all possible interactions between 2, 3 and 5).

Host plant vigour

For the cabbages, we estimated the vigour in terms of total plant dry mass at the end of the experiment. Plant dry mass is an indication of plant fitness that is the responses of cabbages to infestation by aphid genotype, in the absence and presence of onion and potentially competing cabbage plants. Plants were oven-dried for 3 days (9 h per day) at 60°C.

From the total plant dry mass, we derived the shoot-to-root ratio for each plant. Plants growing in a steady state tend to conserve the same shoot-to-root ratio (Thornley, 1972; Guilbaud *et al*., 2015), while plants in competition may be incentivized to allocate more mass to root (Gersani *et al*., 2001). Herbivory by aphids may also affect this ratio as they prevent a steady-state growth (Guilbaud *et al*., 2015). We, therefore, assume shoot-to-root ratios can help us further our understanding of plant–plant as well as plant–aphid interactions.

Here, we used a GLM with a Gaussian family after the response variable was log-transformed (running the model with a quasi-Poisson family without data transformation yielded the same pattern of results). The explanatory variables were: (1) onion (0 = [0 meaning four host plants (cabbage) per microcosm and onion plants are absent] that is the baseline of this effect [0.25 meaning three host plant (cabbage) per microcosm with one onion only present] and [0.5 meaning two host plants (cabbage) per microcosm with two onion plants present]). This effect reflects the proportional numerical host plant to repellent plant differences per microcosm. (2) Cabbage genetic variability (categorically: uni-mix1 [savoy cabbage] that is the baseline of this effect, uni-mix2 [red cabbage], di-mix [savoy-red cabbages]. (3) Aphid effect (aphid absence [baseline], Pink, Green, mix of Pink and Green). (4) Aphid interaction mode (alone [baseline], competing).

Data are available via Figshare repository: https://figshare.com/s/bfb8364d10065ec11927.

Results

Aphid performance, behaviour and polyphenism were affected by the different layers of our experiments. The analysis of the interactions of the different factors revealed a complex scene where competition, genetic variability of aphid and cabbage as well as variable nuisance by onion, were influential in shaping the observed outcomes. Only the significant results are shown in this section. See the corresponding Supplementary Materials (mentioned below) for further details.

Aphid's standpoint

Performance

The performance of aphids depended primarily on aphid genotype ($\chi^2 = 49.56$, df = 1, $P < 0.0001$), with the Green aphid generally performing better than the Pink one (fig. 2). The cabbage genetic variability affected aphid performance ($\chi^2 = 26.37$, df = 2, $P < 0.0001$) with variable aphid population size dependent on context. Aphid interaction mode ($\chi^2 = 27.69$, df = 1, $P < 0.0001$) also strongly influenced aphid performance. Onion effect (repellent plant to host plant availability) was also significant ($\chi^2 = 7.85$, df = 1, $P = 0.005$). Additionally, aphid performance was also affected by the plant shoot-to-root ratio ($\chi^2 = 5.13$, df = 1, $P = 0.024$). Further, the interaction of cabbage genetic variability with aphid genotype was strongly significant ($\chi^2 = 47.8$, df = 2, $P < 0.0001$), as the Green aphid performed alone better than the Pink aphid on red cabbage. Whereas the Pink performed alone better on savoy cabbage; note that the Green had the upper hand in every competition scenario across all contexts (fig. 2). The interaction between cabbage genetic variability and aphid interaction mode was also significant ($\chi^2 = 11.59$, df = 2, $P = 0.003$), with competition being harsher on the Pink aphid clone on red cabbage and on the cabbage di-mix than on savoy cabbage. The interaction between aphid genotype and their interaction mode was also significant ($\chi^2 = 10.12$, df = 1, $P = 0.001$). Moreover, onion vs. cabbage availability affected the Pink and Green aphids differently ($\chi^2 = 5.26$, df = 1, $P = 0.022$). This may indicate that one genotype reacted more strongly to the embedded environment compared with the other. Further, the three-way interaction between onion effect, cabbage genetic variability and aphid interaction mode was significant ($\chi^2 = 15.77$, df = 2, $P = 0.0004$). We observe that Pink aphid tolerated onion nuisance as its population soared Savoy cabbage in marked contrast to its performance on red cabbage. This was contrary to Green aphid performance which greatly increased on red cabbage and strongly decreased on red cabbage. The interaction between onion, aphid genotype and aphid interaction mode was significant ($\chi^2 = 8.92$, df = 1, $P = 0.003$). So was the interaction between cabbage genetic variability, aphid genotype and aphid interaction mode ($\chi^2 = 17.91$, df = 2, $P = 0.0001$). The four-way interaction between onion, cabbage genetic variability, aphid genotype and aphid interaction mode was also significant ($\chi^2 = 6.9$, df = 2, $P = 0.032$). We note that none of the remainder possible interactions were significant. See Supplementary Materials, table S1 for detailed model results on the levels comprising the main effects in question, table S2 for a post-hoc Tukey test of performance differences across contexts and table S3 for complementary infographics on aphid genotype performance under variable onion nuisance and cabbage genetic variability.

Percentage off-plant

The highest number of aphids off-plant (10.1%) in the absence of onion was recorded on the cabbage mix for the Pink aphid, while the highest percentage (8.6%) onion was recorded for the Green aphid on the cabbage mix where onion was present. However, in general aphid proportions off-plants were mainly affected by the onion effect ($F_{(1,96)} = 28.34$, $P < 0.0001$), which increased the propensity of aphids to leave the plants. The effect of plant shoot-to-root ratio was also significant ($F_{(1,97)} = 9.44$, $P = 0.003$), but aphid genotype by aphid interaction mode was only marginally significant ($F_{(1,83)} = 3.411$, $P = 0.069$) (fig. 3). See Supplementary Materials, table S4 for detailed model results on the levels comprising the main effects in question, and table S5 for post-hoc Tukey pairwise differences of the effect of onion effect on off-plant aphid distribution. Our observations and analyses indicate that onion nuisance and the biomass of cabbages (food availability) played the major role in the induction of aphids to leave the plant. The onion effect is seen in fig. 3 with greater numbers of aphids off-plant under onion nuisance.

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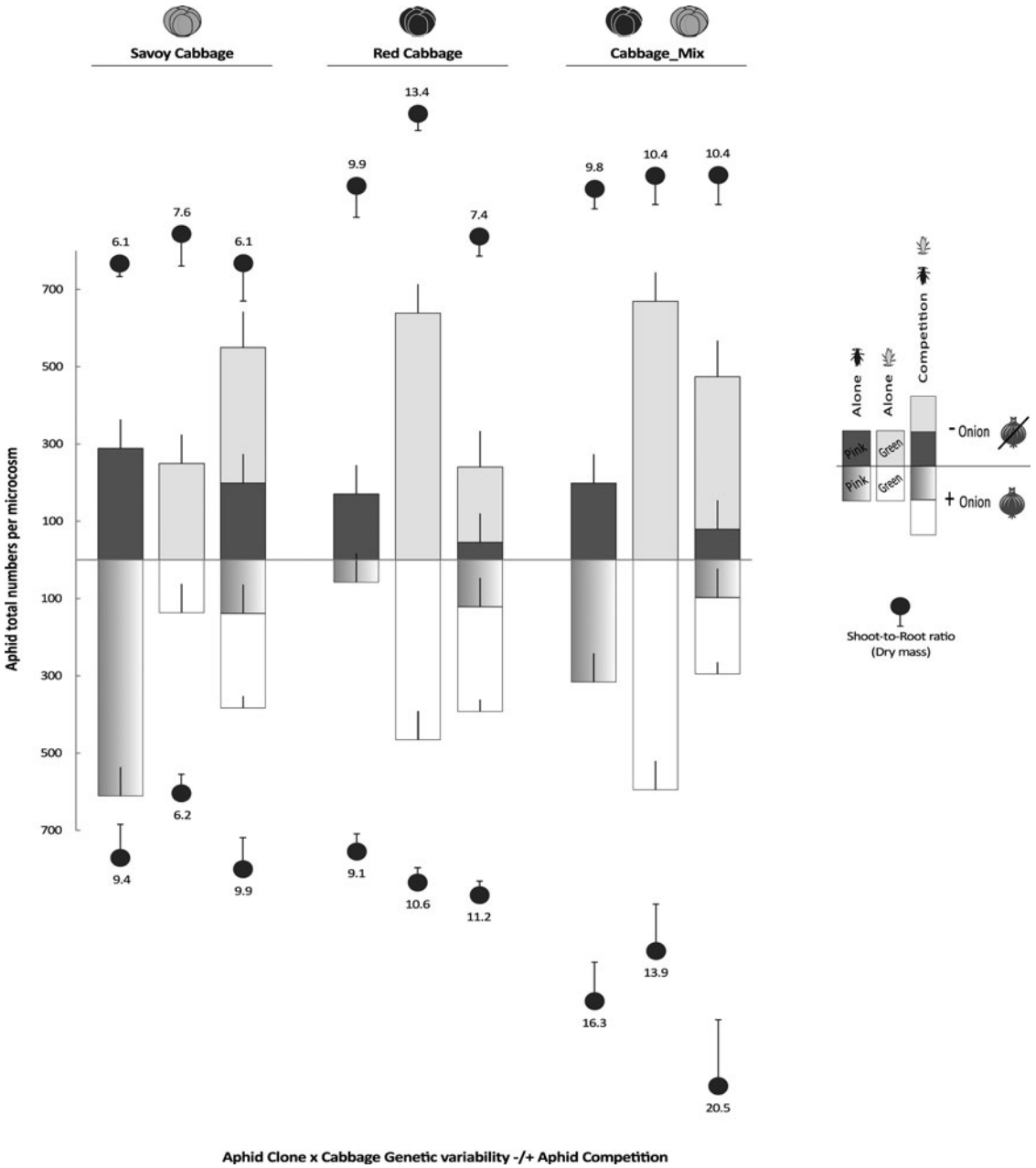


Fig. 2. Aphid performance. Average aphid total numbers per microcosm (±SE) are displayed. With onion as a nuisance factor, two potato peach aphid clones (Pink, Green) performed alone and under competition on each of the respective cabbage mixes. Uni-mixes were composed of savoy cabbage or red cabbage, with or without onion replacing one cabbage plant in each of the uni-mix cultivations. For the cabbage di-mix (savoy and red together) two onion plants were employed instead of two cabbages (one seedling savoy and one seedling red cabbage). In each microcosm there were always four plants. All seedlings were grown at opposing points close to the edges of the pot.

Alatae

The proportions of alatae produced depended on the onion effect ($F_{(1115)} = 8.58$, $P = 0.004$), cabbage genetic variability ($F_{(2113)} = 4.14$, $P = 0.019$) and aphid interaction mode (alone or in competition) ($F_{(1112)} = 14.65$, $P = 0.0002$). The shoot-to-root ratio had also a highly significant effect on alata production ($F_{(1116)} = 7.61$, $P = 0.007$). As for the interactions, only onion effect by cabbage genetic variability was significant ($F_{(2110)} = 3.1$, $P = 0.049$); onion effect by aphid interaction mode was marginally significant ($F_{(1109)} = 3.93$, $P = 0.05$), while none of the remainder possible interactions were significant. More alatae were produced on red cabbage compared with savoy cabbage (fig. 3). Further, when performing alone, winged morph production by Pink aphid was lacking on all cabbage mixes, while Green aphid was always induced, under the effect of onion, to produce winged

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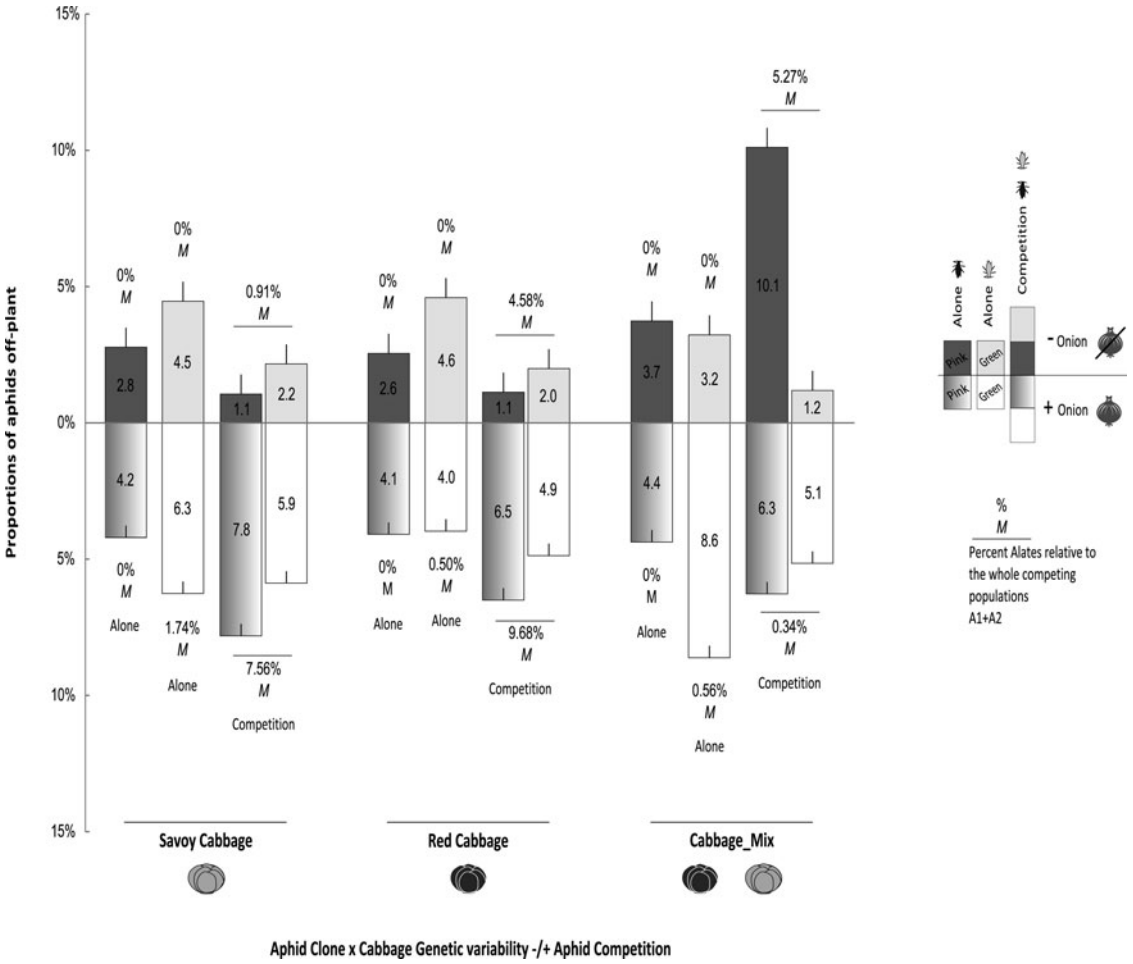


Fig. 3. Aphid off-plant distribution and production of winged morphs (%*M*). An illustration of the average percent of aphids counted off their host plants per microcosm ±SE under onion effect, two potato peach aphid clones (Pink, Green) performed alone and under competition on the respective cabbage mixes. Uni-mixes were composed of savoy cabbage or red cabbage, without or with onion presence replacing one cabbage plant for each of the uni-mix cultivations. For the cabbage di-mix (savoy and red together) two onion plants were employed instead of two cabbages (one seedling savoy and one seedling red cabbage). All seedlings were grown at opposing points close to the edges of the pot. Proportional count of the alatae is related to each treatment line. Under aphid competition, percent of the total morphs belonging to both clones was calculated all together.

offspring in these contexts. This suggests that the Green might have majorly contributed to the percentage of alatae under aphid competition, which was remarkably high on the red cabbage uni-mix (fig. 3).

Plant's standpoint

The number of cabbages that could be infested by aphids differed as well as the level of nuisance by onion on a basis of cabbage genetic variability. In the absence of onion, there were four cabbage plants to infest and zero repellence, the uni-mix culture with onion had three cabbage plants and one onion plant (repellence), while the cabbage di-mix with onion had only two cabbages and two onion plants (heightened repellence). This numerical difference naturally increased the aphid pressure on cabbage, when compared with the cases where onion was absent, but it also increased the space – and resources – available for growth for the cabbages. This resulted in significant effects of onion (likelihood ratio [LR] $\chi^2 = 10.59$, df = 1, $P = 0.001$), aphid genotype (LR $\chi^2 = 18.11$, df = 3, $P = 0.0004$) and aphid interaction mode (competing or performing alone) (LR $\chi^2 = 3.9$, df = 1, $P = 0.048$) on the final dry mass of cabbages. The effect of cabbage genetic variability was not significant. See fig. 4 and Supplementary Materials, table S7 for model details and the significance of the levels comprising the main effects and tables S8–S10 for post-hoc Tukey multiple comparisons. It is notable that aphids showed a very strong propensity to avoid contact with the onion and thus by default no colonization of any onion seedlings occurred during the course of the experiment. Further, in Supplementary Materials note 'Aphid effect on host plant vigour revisited' including, tables S11 and S12, we provide complementary details on the analysis of host plant vigour.

Discussion

The performance of aphids was largely dependent on intrinsic genetic qualities, their growing environment and the

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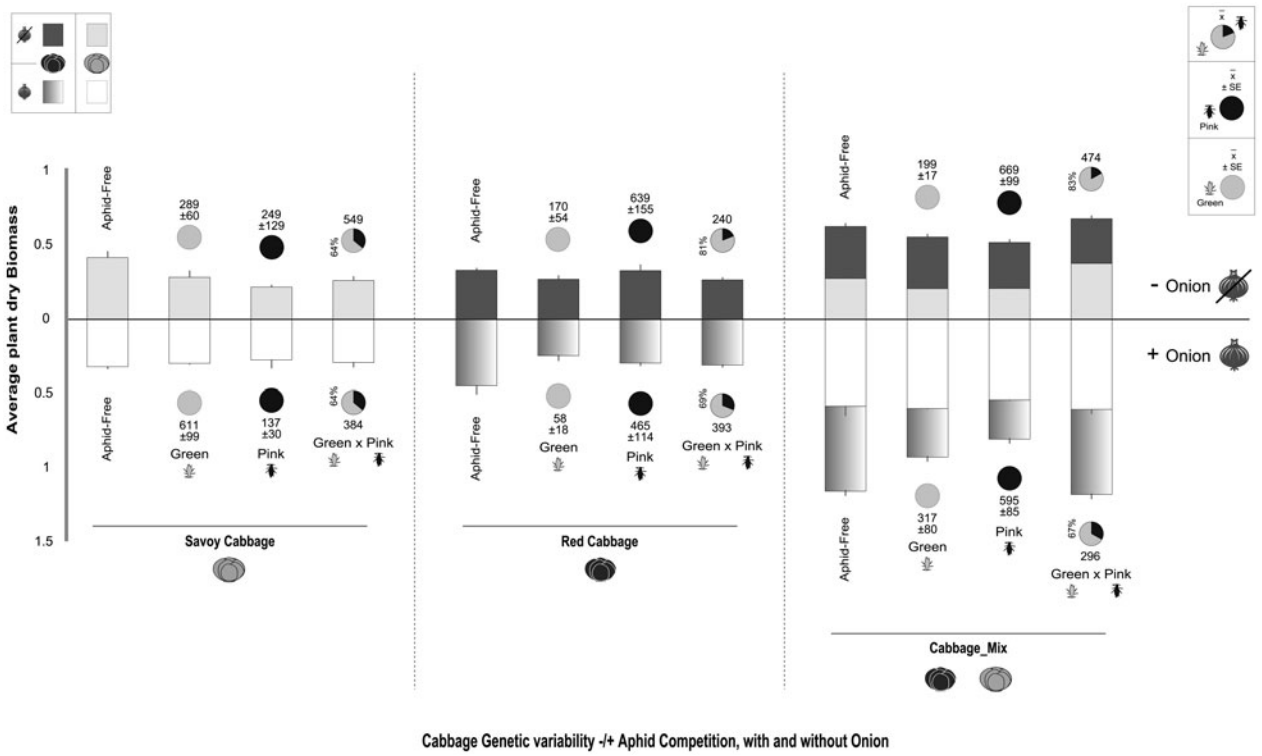


Fig. 4. Plant vigour. The average total plant dry mass (in g) per microcosm (±SE), under onion effect, is depicted illustrating plant response to the different contexts of aphid absence and aphid genetic variability and interaction mode. The two potato peach aphid clones (Pink, Green) were absent or performed alone or under competition on three respective cabbage mixes. Uni-mixes were composed of savoy cabbage or red cabbage, without or with onion presence replacing one cabbage plant for each of the uni-mix cultivations. For the cabbage di-mix (savoy and red together) two onion plants were employed instead of two cabbages (one seedling savoy and one seedling red cabbage). All seedlings were grown at opposing points close to the edges of the pot. The plant dry mass bars are annotated with average aphid total numbers (circles in the case of aphids performing alone, and pie charts in the case where aphids were in competition); the percent next to the pie chart of aphid competition points to the Green aphid's proportion of the whole average of mixed aphid population.

interaction of those two factors. In this study, each aphid genotype preferred a certain cabbage cultivar to feed upon, and both were generally repelled by onion relative to host plant availability and variability. Our results also clearly show that the environmental setup affected the vigour of cabbages which suggests that the functional genetic variation/diversity of the environment can be linked with the productivity of the plants and the performance and/or effects of interacting plant–parasite conspecifics, but this might vary by context of interaction, especially when an enemy of the pest (onion herein) acts as a biotic stressor of the pest. Mastering these diversity effects in agricultural management could lead to mechanism-based agricultural developments that we will discuss below.

Aphids

The Green aphid was the better performer and competitor in this experiment, able to effectively surpass the population growth of the Pink aphid conspecific; as intraspecific competition possibly contributed to the suppression of the Pink numbers. This effect, in the absence of onion, was particularly strong on the red cabbage and the di-mix, where the Green aphid grew best. The Green aphid's population growth was negatively affected by onion, when compared with its Pink counterpart, but this was not the case for the Pink aphid as on savoy cabbage, the onion effect actually resulted in a dramatic improvement of the performance of this aphid conspecific, contrary to the pattern observed on red cabbage. For both aphids, the mixed environment with both red and savoy cabbages resulted in performance approximately midway of both single treatments. These results strongly suggest that aphids have nuanced 'personalities' *sensu* Schuett *et al.* (2011), with large display of plasticity depending on the embedding environment (Tremmel & Müller, 2013). A possible expression of these aphid personalities may be found in the differential performance on different cabbages that can be driven by the variance in sulphur and glucosinolate production between savoy and red cabbages (Cao *et al.*, 2018). Kanvil *et al.* (2014) showed a similar analogy with pea aphid biotypes reared on various *Medicago truncatula* genotypes; and this also receives support from our previous experiment with a group of pea aphid clones (Khudr *et al.*, 2017*b*) showing a large array of contextual performance and behaviour on different cultivars of *Vicia faba*.

Propensities to leave the host plant, in the current work, were inducible by crowdedness and/or onion presence. However, interestingly, there was a higher probability for the Pink aphid to leave the plant in the mixed environment without onion compared with any other contexts. The variable presence of onion not only increased the proportions of aphids off-plants, but also it seemed to trigger the generation of

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winged-morph aphids. Pahla *et al*. (2014) described a similar effect with *B. brassicae* on rapeseed plant (*B. napus*). This increase in aphids off-plants may be a direct result of nuisance/repellence by onion, or an indirect effect where onion presence triggered a change in the volatile profile of the cabbage as shown in potato plants that were exposed to onion (Ninkovic *et al*., 2013; Dahlin *et al*., 2015). But, it was competition between aphids, and perhaps subsequent crowding (Powell *et al*., 2006), that could accelerate the depletion of available resources given the exponential reproduction of aphids. That probably was the biggest factor explaining the production of alatae in our study. Again, aphid competition and onion nuisance differentially increased the fraction of winged-morphs, while the highest percent of alatae was observed under competition on red cabbage (subject to variable onion nuisance).

The role of onion in our experiment was straightforward: overall, the onion negatively affected the performance of aphids and played a significant role in triggering aphids to leave the cabbage hosts. But the onion differentially affected the two genotypes of aphid (Green was more affected than Pink). The onion also differentially affected the vigour of the plants with the strongest effect of onion on plant vigour observed in mixed settings. Onion is a well-known companion plant that acts as a natural insect repellent (Dancewicz & Gabryś, 2008). It is successfully used as a plant companion in developing countries that do not rely heavily on agricultural machineries (Altieri, 2012; Parker *et al*., 2013; Sallam, 2013). Furthermore, Haan & Vasseur (2014) showed that root competition between lettuce and onion benefited the lettuce at the expense of the onion. In our experiment, we see that the presence of onion tended to decrease the shoot-to-root ratio, which may be an indication of competition for space and nutrients belowground. It may also be an indirect effect of aphid infestation in microcosms with fewer plants to infest and thus leading to a bigger aphid loads on limited aboveground plant biomass. Comparing fig. 3 and fig. 4, it appears that onion nuisance and/or lower plant biomass is generally linked to larger numbers of aphids off-plant that was not necessarily intensified by crowding. For example, proportions of Pink aphid congeners off-plant on uni-mixes (savoy cabbage red cabbage) were almost identical and became heightened when onion was present, although the population of the Pink sharply differed in size on each of the uni-mixes. In this regard, Green aphid showed noticeable differences to the Pink on the cabbage uni-mix contexts. The different patterns of abundance/performance and off-plant proportions of Pink and Green aphids on cabbage di-mix could be plausibly attributed to a genotype by mode of interaction effect.

Nevertheless, intercropping methods may need to be explored to assess the optimal cropping pattern of onion and the other main crops. Sulvai *et al*. (2016), for example, showed that enclosing lettuce with a continuous line of onion repelled the caterpillar *Agrotis ipsilon* more effectively than was reported for monoculture. Zhou *et al*. (2013) emphasized the utility of garlic *A. sativum* as pesticide alternative in polyculture for suppressing grain aphid *S. avenae* population. Furthermore, Ninkovic *et al*. (2013) showed that polyculture with onion modified the volatile profile of potato plants, leading to lower infestation by aphids. In our experiment, the effect of onion was likely magnified by the fact that the microcosms were closed, yet meshed, leaving the aphids with no choice to diffuse, and hence this suggests that onion can be an effective bio-control agent in greenhouse settings. However, the integration of onion may not be the one-size-fits-all solution against aphid infestation. Functional diversity is a context-dependent process where all interacting parties influence the outcome. For example, in our experiment, the Pink aphid performed better on savoy cabbage when the onion was present. The addition of the Green aphid negatively affected the performance of the Pink aphid and not *vice versa*. In particular, the addition of the Pink aphid in the savoy cabbage uni-mix with onion actually improved the performance of the Green aphid. Thus, a thorough knowledge of the interaction matrix of all involved interactors is necessary in order to shape and diversify an agroecosystem towards a desired outcome. Although in general, intercropping is advantageous, unguarded use of diversity may backfire e.g., in terms of intercrops incompatibility and added cost plus complications related to lacking specialized machinery and context-dependent differential watering requirements and possible subsequent yield loss (Lithourgidis *et al*., 2011; Gebru 2015). Also, prompted diversity in the agricultural plot may induce undesired higher performance of herbivores as a byproduct of diversity-related increased plant productivity (Kotowska *et al*., 2010). However, the advantage of intercropping with onion is that the latter is not a good diet for phloem-feeders and thus the onion companionship harnesses the benefits of diversity and plant vigour *plus* to productivity (Kotowska *et al*., 2010; Wu *et al*., 2016), whilst herbivores remain under control.

Plants

In addition to the observation of aphid performance and behaviour, we tracked the vigour of cabbages. Here, again, the context affected the outcome and, unsurprisingly, the presence of aphids decreased the overall plant vigour, but that was less apparent in the red cabbage microcosms. The presence of onion in single plots without aphids decreased the final dry mass of cabbages, implying that the plants were somewhat competing.

In mix plots however, the combined dry mass of red and savoy cabbages surpassed that of the uni-mixes and this effect was magnified when onion was present; such a facilitation effect is not rare in diverse settings (Brooker *et al*., 2008; Haan & Vasseur, 2014). In particular, nearly similar plant dry masses were shown on the cabbage uni-mixes without onion, and the same dry mass was observed for the uni-mixes when both aphid genotypes were present. We also argue that onion presence, albeit being numerically variable, had a stabilizing effect on the dry mass of savoy cabbage in uni-mix situations; an effect also observed in tomato (Wu *et al*., 2016). As such, the variable effect of onion as a companion plant on the main crop's vigour especially for the di-mix cases, even when aphids were present, can be advantageous for the cabbage and may apply to management of aphid infestation due to the anti-insect repulsive effect of onion plus to its positive effect on cabbage vigour in genetically variable contexts (Hooks *et al*., 2007; Wu *et al*., 2016; Stenberg, 2017). These findings are important because they help show that aphid competition and plant facilitation act in concert and may thereby neutralize the negative impact of aphid infestation and plant–plant space competition on the main crop vigour (see Zhang & Li 2003).

Counter-intuitively, adding another aphid genotype to an already infested plot by a different aphid genotype might effectively decrease the damages done to the plant. We could, therefore, speculate that any advantage arising from competition between aphids to the benefit of their shared host plant

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can be utilizable in pest. Since young leaves are more vulnerable to aphids, which prefers to feed on them (Cao *et al.*, 2018), compared with older leaves, the earlier aphid competition occurs, the better. Given the exponential rate of aphid reproduction, this could delay catastrophic consequences in agricultural settings.

Here, we can start to describe one mechanism for the diversity-productivity gradient, a first step towards an effective modelling of such effects with direct use in agricultural settings. Onion enhances the growth of cabbage because of asymmetrical plant–plant interaction, while at the same time releasing chemical compounds that can confuse or repel aphids (e.g., Wu *et al.*, 2016). A second layer of environmental effect is caused by the presence of two cabbage cultivars with different attraction levels for genetically variable aphids, which can lead to a context-specific resource dilution effect (Otway *et al.*, 2005), mitigating pest burden on the plant by reducing the numbers of aphids aggregating on a specific plant cultivar in the genetically variable plot. Finally, a third layer of environmental complexity is created by the competition between aphid conspecific genotypes that negatively affect the fitness of them both (Khudr *et a*l., 2014). The clear winners of this multi-layered game are the cabbages that achieved a cumulated mass in this situation that is tantamount to the case of a non-infested plot.

This novel result extends our experimental study to the larger spectrum of functional diversity, and proposes a mechanical explanation for the observed diversity-productivity gradient. However, much work remains to be done because the interactions between the different elements of an ecosystem are not fixed in time and eco-evolutionary dynamics should be taken into account, if we want to be able to accurately predict the long-term state of agricultural systems (Whitham *et al.*, 2003; Utsumi *et al.*, 2011). These dynamics can take place quickly, when selection pressures are great (Delong *et al.*, 2016); the emergence of pesticide-resistant insects is a direct result of such pressures (Baidoo *et al.*, 2012).

Concluding remarks

Genetically variable systems are more stable and could drive down the consumption of pesticides in the developed and the developing worlds alike. More diverse systems, even including enemies of the focal crop's enemies, can facilitate the burdensome task of controlling insecticide-resistant generalist phloem-feeding insects. Our findings describe a complex as well as a context-dependent variation in the fitness of host plant and pest across a variety of genetically and ecological variable treatments. We argue that multi-layered contextual experiments are a useful first step to delineate a mechanistic framework that would allow the clever and profitable use of eco-evolutionary dynamics across different species in agricultural settings. Specifically, companion planting effects, as interspecific genetic variation effects, can be integrated into the effects of intraspecific genetic variability in farming, whilst contributing an added yielded benefit to the main crop profit. Indeed, companion planting can act as a bridge between agricultural practices and ecological research. More research is needed to understand the interplay taking places in these diverse systems at the genetic, spatial, ecological and evolutionary levels in larger agricultural settings. This understanding could serve as vital part of the foundation for a healthier and greener future necessary to adequately feed billions of humans.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S0007485319000373

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Authors contribution

CG drafted and abbreviated the article, enriched and compiled the bibliography and provided insights into the optimization of data analysis and visualization. MSK designed, did the experimental work and performed the data analysis and visualization. MSK also produced the skeleton of the writing up and contributed to the enrichment of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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