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An aphid pest superclone benefits from a facultative bacterial endosymbiont in a host dependent-manner

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Research Article

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Title Page:

An aphid pest superclone benefits from a facultative bacterial endosymbiont in a host dependent-manner

Running title: *Host dependent benefit of aphid endosymbionts*

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Abstract

The English grain aphid, *Sitobion avenae*, is a significant agricultural pest affecting wheat, barley, and oats. In Chile, the most prevalent and persistent clone (superclone) of *S. avenae* harbours the facultative endosymbiont bacterium *Regiella insecticola*. To determine the role of this bacteria in the ecological success of this superclone, the presence of *R. insecticola* was manipulated to evaluate the impact on 1) the reproductive performance of this clone in two host plant species (wheat and barley), 2) the production of winged morphs, 3) changes in the proteomic profile of insects, and 4) root/shoot ratio of plant. It was determined that this superclone of *S. avenae* proliferates differentially in the host plants studied depending on the presence of the facultative bacterial endosymbiont, observing that the clone develops better in wheat when it is infected with *R. insecticola* while the opposite occurs when it develops in barley. Aphid biomass was higher when harbouring *R. insecticola*, particularly in barley. Individuals infected with *R. insecticola*, in both host plants, showed higher proportion of winged individuals. The protein regulation of aphids on wheat was comparatively lower and stable than that on barley. A higher root/shoot biomass ratio was detected in wheat than in oats in plants attacked with aphids harbouring *R. insecticola*. *R. insecticola* significantly affects the reproductive and proteomic performance of the *S. avenae* superclone, changes influenced by the host plant, suggesting that the host plant x facultative endosymbiont interaction can drive host specialization intraclonally, partly the ecological success of the superclones.

Key words: host specialization; endosymbionts; aphid population growth rate; insect proteomic.

Research Highlights

- The aphid endosymbiont *R. insecticola* affect the reproductive performance of *S. avenae*
- *R. insecticola* alter the aphid reproductive performance in a host-dependent manner

- *R. insecticola* showed a positive effect on wheat and a negative on barley

1.INTRODUCTION

Aphids (Hemiptera: Aphididae) are important crop pests that amplify clonally under favourable conditions. There is an increasing number of studies describing the presence of few widespread multilocus genotypes ("clones" for simplicity) within aphid species, particularly invasive ones with outstanding ability to persist in time and space (called "superclones") (Vorburger et al. 2003; Fenton et al. 2010; Harrison and Mondor 2011; Piffaretti et al. 2013; Chen et al. 2013; Nibouche et al. 2014; Harris-Shultz et al. 2017, 2022; Zepeda-Paulo et al. 2017a; Rubio-Meléndez et al. 2019). However, few studies have addressed the causes explaining this ability. Hypotheses explaining the success of superclones that have found support, states that they exhibit: 1) greater polyphenism enabling responses to variable environmental factors such as host plant quality (Castañeda et al. 2010a), 2) obligate asexuality enabling superclones to do not spend energy on finding suitable mates or mating sites (Piffaretti et al. 2013), 3) adaptation to their host plants (Fenton et al. 2010), 4) the ability to tolerate allelochemicals (Castañeda et al. 2010b), and 5) flexibility on feeding behaviour allowing a similar reproductive performance across different context (Barrios-SanMartin et al. 2016a). Surprisingly, the role of facultative bacterial endosymbionts on the ecological success of these invasive superclones has not been studied yet.

The grain aphid *Sitobion avenae* is a worldwide aphid-pest of cereals. While populations in UK, north of France and Romania are predominated by cyclical parthenogenetic asexual lineages and typically featured by a high genetic diversity (Sunnucks et al. 1997; Dedryver et al. 2001; Papura et al. 2003; Llewellyn et al. 2004), populations in Australia, Chile and China (the introduced range) are predominated by a dissimilar number of obligated parthenogenetic asexual lineages depending on the region (Wilson et al. 1999; Figueroa et al. 2005; Xin et al. 2014). In Chile, this aphid was most probably introduced in the middle of 1970s from Europe (Apablaza and Fernández 1982; Figueroa et al. 2005), and it has been shown that nearly 90% of its genotypic variation is accounted for by only four predominant asexual lineages (Figueroa et al. 2005; Zepeda-

Paulo and Lavandero 2021). One of these asexual lineage dominates rapidly early in the season and displays low variance in performances on different hosts (Castañeda et al. 2010b; Zepeda-Paulo and Lavandero 2021). Interestingly, asexual lineages of *S. avenae* in Chile varied in the presence of the facultative bacterial endosymbionts (Sepúlveda et al. 2014), with the most frequent asexual lineages found to be regularly harbouring *Regiella insecticola* (Enterobacteriales, Enterobacteriaceae)(Zepeda-Paulo et al. 2017). Since *R. insecticola* has been reported to improve the fitness in a host dependent manner in the pea aphid (Tsuchida et al. 2004), it is likely that this bacterium plays a role in the performance of *S. avenae*, determining ecological success on different host plants.

Here we report a study with the most common genotype of the aphid *S. avenae* in Chile, in which the presence of the predominant facultative bacterial endosymbiont *R. insecticola* was manipulated to assess the impact of this endosymbiont on the reproductive performance of this aphid on these two host plants (wheat and barley), and the changes that this generates in the whole-body proteomic profile of aphids. The latter can shed light on the physiological mechanisms underlying reproductive performance in each host due to the presence of this facultative endosymbiont. These two hosts were chosen because they are contrasting in terms of the prevalence of *S. avenae* in Chile (Figueroa et al. 2005; Sepúlveda et al. 2017), since while it is very recurrent in wheat it is scarce in barley, a situation that allows evaluating, controlling for the genetic background, the impact of harbouring the facultative endosymbiont *R. insecticola*. Plant responses (root/shoot ratio) were also measured to assess the consequences of endosymbiont-dependent aphid herbivory.

2.MATERIAL AND METHODS

2.1 Aphids and plants

Individuals of the most widely distributed genotype in cereal crops in the Maule to De Los Rios regions of Chile of *S. avenae* were kindly provided by

Dr. Francisca Zepeda-Paulo. This genotype was characterized with eight microsatellite loci (*Sm11*, *S3.43*, *S16b*, *S30*, *S4Σ*, *S5L*, *Sm17* and *Sm10*) and corresponds to the genotype G as described by Zepeda-Paulo et al. (2021) found to harbour *R. insecticola* (see more details in Zepeda-Paulo et al. 2021). In the present study we used this genotype as two asexual lineages: one infected (hereafter E+) composed by individuals naturally harbouring the facultative endosymbiont *R. insecticola* (as collected from the field), and the other asexual lineage was originated by individuals treated with antibiotics (ampicilin, cefotaxim and gentamicin) by using the method of (i) artificial diet (synthetic diet) and (ii) micro-injections with the methods of Koga et al. (2007) and Simon et al. (2011) resulting in *R. insecticola*-disinfected individuals (hereafter E-). Both lineages harboured the primary endosymbiont *Buchnera aphidicola*. Aphid colonies (E+ and E-) were reared in separate mesh cages (50 cm x 42 cm x 31 cm) containing pots with wheat or barley seedlings. Thus, four distinct colonies of the genotype G1 were reared in the laboratory.

Host plants used were barley (*Hordeum vulgare* L., cultivar Sebastian) and wheat (*Triticum aestivum* L., cultivar Pantera). Plants were grown in organic soil during one week in a greenhouse by sowing about twenty seeds per pot (bottle cap in pots). Every pot was disinfected (Virginia igenix) and fertilized (Nutrifeed follare) every week. The growth chamber was under controlled conditions (22 ± 1 ºC, 16:8 photoperiod).

2.2 Aphid reproductive performance and the effect on host plants

To use age-synchronized aphids for the experiments with E+ as E- aphids, firstly 50 adult aphids were placed on potted wheat or barley plants. After 12 h, all adults were removed, leaving only new nymphs on plants. Four days later, four wingless aphids were transferred to each individually potted wheat or barley seedlings. In total, 20 pots with wheat seedlings with E+ aphids, 20 pots with wheat seedlings with E- aphids, 20 pots with barley seedlings with E+ aphids, and 20 pots with barley with E- aphids were obtained, each selected for setting the experimental systems. After 14 days the final number aphids, including winged individuals was

recorded. With the aim of determining relations in aphid weights, 25 wingless adults were collected in each of the four rearing boxes. They were weighted with an analytic balance (XP2U Mettler Toledo, Columbus, OH, USA, precision: 10^{-3} mg). In addition, once the reproductive performance experiment finished, the plants were removed from the pots and carefully separated the root and shoots, dried out for about one week at 60 °C and (Memmert Beschickung-Loading Modell 100-800) and weighted with an analytical balance (Radwag as 220/c/2).

2.3 2D-DIGE and protein identifications

Aphids hosting or not a facultative symbiont, namely *R. insecticola*, reared on two host, wheat or barley were collected to perform proteomic analysis. Whole body proteins from around 50 mg fresh *S. avenae* asexual lineages were extracted, purified, and quantified as described by Francis et al. (2010). Three Cy dyes (GE Healthcare) were used for labelling and protein samples of aphid asexual lineages were labelled either with Cy3 or Cy5 and mixed with an internal reference standard protein mixture (pooled from equal aliquots from all experimental samples) labelled with Cy2. Two replicates from each treatment with one dye (Cy3 or Cy5) and a third replicate with the other of the two Cy dyes were established for a conventional dye swap of DIGE. The first and second-dimensional electrophoresis, the excision of protein spots and the process of protein identification were performed following the description in Francis et al. (2010). The Mascot server 2.2.06 with BioToolsTM3.2 (Bruker Daltonics) and NCBI (National Center for Biotechnology Information) server accessed accessed in November 2022 were used for protein identification. The identified proteins were categorized according to metabolic function using the Kegg pathway database (http://www.genome.jp/kegg/pathway.html, accessed in November 2022) and Expasy Proteomic tools (http://www.expasy.org/tools/, accessed in January 2022).

2.4 Statistical analysis

The total number of aphids (i.e., nymphs + adults + adults winged) and adults winged were subjected to *glm* with Poisson distribution and log-link, whereas the aphid weight and plant root/shoot ratio were subjected *glm* function with a Gaussian distribution as the error structure. The *glm* included the factor “endosymbiont” with the levels E+ and E-, and the factor “host plant” with the levels wheat and barley. After detecting a significant effect, the Tukey *post hoc* comparison with Šidák correction procedure was used to make pairwise contrasts between treatments using the *emmeans* package (Lenth R et al. 2021). Wilcoxon rank sum test was used to compare fold ratios of protein regulation. All statistical analyses were carried out with the R software, version 4.2.2 (2022; R Development Core Team).

3.RESULTS

3.1 Effect of the presence endosymbiont on aphids’ performance and plant growth

The total number of aphids at the end of 14 days varied depending on the host plant and the presence of the endosymbiont, as revealed by the significant interaction between the endosymbiont and host plant factors (Deviance= 783.9; d.f.= 1, 76; p <0.001). In wheat, the final number of aphids was higher in the E+ aphids, while in barley, the opposite was observed, with a higher number of aphids in the E- aphids (Fig 1A). The final number of winged individuals was very low, but was affected only by the endosymbiont factor, with winged individuals only found in the E+ aphids in both host plants (Wheat: E+=0.65 ± 1.04 and E-: 0 ± 0; Barley: E+=0.30 ± 0.57 and E-: 0.05 ± 0.22; Deviance= 19.3; d.f.= 1, 78; p <0.001). Aphid final weight exhibited a significant effect of the endosymbiont factor (F= 214.7; d.f.= 1,96; p <0.001; Fig. 1B) with E+ aphids being weightier than E-. There was also a significant effect to the host plant factor (F= 49.2; d.f.= 1,96; p <0.001), with aphids being weightier on barley than on wheat. Additionally, there was a significant interaction between the endosymbiont and host factors (F= 5.0; d.f.= 1,

96; p <0.05), with both E+ and E- aphids being weightier on barley than on wheat. On the other hand, the root/shoot ratio also varied depending on the host plant and the presence of the endosymbiont, as revealed by the significant interaction between endosymbiont and host plant factor ($F_{1, 76} = 7.79$; $p < 0.001$; Fig. 1C). In wheat, the root/shoot ratio was higher on plant attacked by the E+ aphids that by E- aphids, while in barley, no difference in the root/shoot was found (Fig. 1C).

3.2 Identification of differentially expressed proteins

In total, 43 proteins were identified from the 2D gel among which spots varied significantly ($p < 0.05$; Figure S1). The complete properties of over- and under-expressed proteins in *S. avenae* asexual lineages with and without *R. insecticola* facultative symbiont on wheat or barley host plants were listed in Table 1. We found that 38 proteins were related to aphids and six6 proteins were related to endosymbionts. The fold change ratios of these proteins between the aphid asexual lineages with or without *R. insecticola* on the same plant, either wheat or barley ranged from 0.27 to 7.7. The absolute range of protein regulation (considering the extremes of up and downregulation fold-ratios) in barley was 1.43 fold-ratio, while it was 0.47 fold-ratio in wheat. With regards to proteins in the aphid group, 58% were detected in *A. pisum* while fewer (8%) were detected in other species (*A. citricidus*, *A. gossypii* and *S. avenae*). With regards to the endosymbiont group, various proteins were detected in *B. aphidicola* (63%), the other were related to Regiella and Rickettsia facultative endosymbionts.

Proteins with different expression levels were relate to several metabolic pathways (Table 1). We found that these proteins accounted for various roles in metabolic pathways such as genetic information processing (20%), cytoskeleton (18%), environmental information processing (16%), energy metabolism (11%), carbohydrates (9%), cellular process and stress responses (both 5%). The presence of *R. insecticola* in aphid developing in barley showed a greater average upregulation of proteins than in wheat (barley fold-ratio = 2.0 ± 1.4, wheat fold-ratio =

1.27 ± 0.28, p<0.001, W = 419, Wilcoxon rank sum test), whereas on wheat infected aphids showed a greater average downregulation than on barley (wheat fold-ratio = 0.88 ± 0.17, barley fold-ratio = 0.57 ± 0.29, p<0.001, W = 75, Wilcoxon rank sum test).

On barley energy and carbohydrate metabolism, as well as cellular process and response to stress were highly downregulated. Contrastingly, downregulation in wheat was globally mild, except in the case of other protein group, particularly in the 1-acyl-sn-glycerol-3-phosphate acyltransferase. Upregulation was globally mild on both host plants, with a distinct high upregulation of the protein 1-acyl-sn-glycerol-3-phosphate acyltransferase on E+ aphids developing on barley.

4. DISCUSSION

In this work we have found that a single genotype of the aphid *S. avenae* proliferates differentially in two hosts depending on the presence of a facultative bacterial endosymbiont. The most striking result of our study was that among aphids sharing the same genetic background, those developing on wheat and harbouring the facultative endosymbiont *R. insecticola* exhibit larger colony development, while the opposite was true for the aphids developing in barley. A similar positive effect of this endosymbiont on reproduction has been found on the Vicia-specialized pea aphid populations (Tsuchida et al. 2004). However, that result was not reproduced in other pea aphid or other aphid species, with studies showing negative or neutral effects of *R. insecticola* on aphid reproduction (Ferrari et al. 2004, 2007; Wang et al. 2016; Luo et al. 2017; Liu et al. 2019). Nevertheless, our results are consistent with field-based monitoring of *S. avenae* in wheat plantations showing *R. insecticola* increased the prevalence throughout time when compared with uninfected individuals (Zepeda-Paulo and Lavandero 2021). It should be noted that G1 genotype dominates early in the season, but a recent study showed that its predominance significantly decreased at mid-season (Zepeda-Paulo and Lavandero 2021). However, although G1 decreases its prevalence during

the season studied, it persists over the years as describe by Figueroa et al.)2005).

Ramírez-Caceres et al. (2019), studying another *S. avenae* genotype (G2) also described a higher population growth of E+ aphids on wheat than on barley, while E- negatively affect the growth of aphids on barley. All this support that *R. insecticola* entail within-genotype difference on *S. avenae* performance across two common food plants, wheat and barley. This suggest that host plant x facultative endosymbiont interaction may drive host specialization even within a genotype. Thus, clones composed by E+ aphid on wheat and E- aphids on barley, are expected to be positive selected and negatively selected, respectively, leading to ecological divergent populations dominated by asexual clones. However, given the temporal instability of the cereal plantations in Chile, these populations might not reach such a divergence (González U et al. 2013). The lack of host-based differentiation of *S. avenae* populations in Chile confirm this hypothesis (Figueroa et al. 2005). Nevertheless, it is surprising that the same facultative endosymbiont can generate such dissimilar effects on its host aphid, which raises several questions about the underlying mechanisms.

Our result show that E+ aphids on both host plants showed higher number of winged individuals, although in a very low number, this contrast with previous studies. For example, E+ individuals of asexual lineages of *S. avenae* from China under crowed conditions, produced less winged offspring than E- aphids (Liu et al. 2019). This result was found at 25 °C, which is slightly higher than our conditions (22 ± 1 ºC). They also found that winged morph production did not showed differences among E+ and E- at higher temperatures, which suggested that winged morph production was dependent on environmental temperature and aphid density. On the other hand, *R. insecticola* negatively affect the production of winged offspring in the pea aphid (Leonardo and Mondor 2006). In our case, because the production of winged individuals in E+ aphids was irrespective of the host plant, this could be an idiosyncratic capacity of this asexual lineage (G1) to respond to the infection with *R. insecticola*.

Remarkably, this asexual lineage is one the most persistent and predominant in the wheat field of Chile (Figueroa et al. 2005) , and frequently found harbouring *R. insecticola* (Zepeda-Paulo et al. 2017) and thus the evolution of this lineage with *R. insecticola* might have reached a fixed pattern of response to this endosymbiont.

Surprisingly, the body weight of E+ and E- aphids did not fallowed the same trend as reproductive performance. Instead, regardless host plant, aphid showed greater weight when they harboured *R. insecticola*. This indicates that the production of offspring is uncoupled with the weight of each individual. This contrast with the results found in *Rhopalosiphum maidis* (Fitch) feeding on barley, where *R. insecticola*-infected aphids performed poorly in weight (Liu et al. 2023). Regarding the distinct effect of *R. insecticola* on weight and reproductive performance in aphids of *S. avenae* sharing the same genetical background, again open questions about the undelaying mechanisms of these responses. Thus, we subsequently develop a proteomic study with the infected and non-infected aphids after their development in wheat or barley, to obtain some light on what the underlying mechanisms are.

Our findings suggest that the presence of *R. insecticola* generates different changes in the proteomic profile of *S. avenae* depending in a host-dependent manner, that could account for the difference in reproductive performance. Interestingly, the fact that protein regulation of aphids developing on wheat was comparatively milder and steadier than on barley, suggest that E+ reared on wheat inflict lower impact of their physiology. In this regard, due to expansion of gene families associated with resistance to insecticides and plant chemical defenses described in the genome of *S. avenae* (Villarroel et al. 2022), if *R. insecticola* would have a beneficial effect on confronting those compounds, a larger regulation of those proteins would be expected when comparing E+ and E- aphids. However, no differential regulation of those proteins was found in our study. Interestingly, this is consistent with the lack of association between the presence of *R. insecticola* and sensitivity to pyrethroids in *S. avenae* population from Germany (Leybourne et al. 2023). Since

populations of *S. avenae* in Chile are most predominant in wheat than on barley (Apablaza and Fernández 1982; Figueroa et al. 2005) and that the genotype G1 is also predominant (Zepeda-Paulo et al. 2017; Zepeda-Paulo and Lavandero 2021), the comparative lower protein regulation on wheat suggests that much steady physiological response as compared with that on barley, is probably due to a recent adaptation of *S. avenae* to wheat after introduction. Indeed, it has been described that *S. avenae* superclones exhibit a broad host range, flat energetic costs for non-induced detoxification enzymes, and low variation in their reproductive performance on different host plants (Castañeda et al. 2010b; Barrios-SanMartin et al. 2016b). The facultative aphid endosymbiont *Serratia symbiotica* manipulates the expression of specific proteins in the pea aphid impairing plant defence response and improve feeding (Wang et al. 2020), a mechanism that may be occurring in E+ in *S. avenae* aphids.

The lower reproductive performance of E+ aphids on barley could be link to the higher number of proteins that were upregulated in these aphids, which could be an indicative of a reaction to a bacterium infection. The upregulation of the 1-acyl-sn-glycerol-3-phosphate acyltransferase enzyme (plsC), which participates several lipid biosynthetic pathways (Chen et al. 2011), and the elongation factor 1-alpha, may both be related with active enzymes delivery as a reaction to *R. insecticola*. However, the functional role of these upregulated protein on E+ aphids remain to be deciphered. On the other hand, downregulation of proteins in E+ on barley such as such as murein hydrolase activator EnvC, a protein produced by *R. insecticola* and normally associated with bacteria proliferation within aphids (Cook et al. 2020), could be aphid defensive response against *R. insecticola* induced by feeding on barley. It remains to be deciphered what are the host-dependent cues triggering such a striking difference in the aphid physiology.

Regarding the effects of E+ and E- aphids on the plants, the higher root/shoot ratio exhibited by E+ aphids compared to E- aphids on wheat plants but not on barley, suggests that these plants experienced a greater response stress. Since plants usually show a higher root/shoot ratio under

water deficiency, it seems that E+ aphids on wheat were more damaging than on E-. It is likely that aphids harbouring *R. insecticola* obtain and specific benefit on wheat which increase their efficiency in the use of this specific plant. This result contrasts with what was found in *Medicago truncatula*, where plants treated with pea aphids free or infected with *R. insecticola* showed no difference on the dry weight of plant shoots (Pandharikar et al. 2020).

5.CONCLUSION

In summary, our laboratory study we detected important effects of *R. insecticola* on *S. avenae* reproductive performance and proteomic which were highly influenced by the host plant. Since these results were detected within one single aphid genotype, the direct impact of the facultative endosymbionts x host plants interaction it is highlighted. Accordingly, as the presence of facultative endosymbionts can alter aphid reproductive performance in a host-dependent manner, we found that the prevalence of facultative endosymbionts, larger on wheat and lower on barley, is also dependent of the host plants.

AUTHOR CONTRIBUTIONS

Claudio C. Ramírez and Frederic Francis conceptualized the research and designed the experiments. Leandro Mahieu and Algélica González-González conducted the aphid performance experiments. María Eugenia Rubio-Meléndez performed the genotyping and endosymbiont detection. Frederic Francis performed the proteomic analysis. Claudio C. Ramírez analyzed the data, prepared the graphic design and wrote the original draft. All authors edited the manuscript.

CONFLICT OF INTEREST

The authors do not have any conflict of interest.

DATA AVAILABILITY STATEMENT

The data supporting this study's findings are available from the corresponding author upon request.

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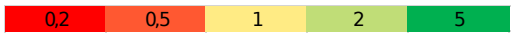
TABLE 1: Identified proteins in *Sitobion avenae* aphids that differ in abundance depending on the occurrence of *Regiella insecticola* (+/-) secondary symbiont on different Barley/Wheat (B/W) host plants.

B+/B- fold ratio	W+/W- fold ratio	Protein identification	Organism	Protein Mw	PI	Mascot score	MS coverage	NCBI reference
Energy metabolism								
		Phosphoglycerate kinase	*Aphis citricidus*	44554	5.6	166	36	gi|52630949
		ATP synthase subunit alpha	*A. pisum*	59986	9.7	111	28	gi|193666827
		ATP synthase subunit beta	*A. pisum*	55777	4.9	72	10	gi|209915626
		Succinyl coenzyme A synthetase	*A. pisum*	34654	8.7	60	15	gi|251823775
		Enolase	*A. pisum*	47492	5.5	152	47	XP_001948161.1
Carbohydrates								
		Phosphoglucosamine mutase	*Escherichia coli*	16527	10.2	67	43	EEV3194948.1
		Glyceraldehyde-3-phosphate dehydrogenase	*A. pisum*	35713	7.7	127	43	NP_001280403.1
		L-xylulose reductase	*A. pisum*	26317	7.66	61	32	NP_001119696.2
		Glyceraldehyde-3-phosphate dehydrogenase	*A. pisum*	35623	7.7	50	3	gi|193688110
Cytoskeleton								
		Actin	*S. avenae*	16788	6.4	118	48	gi|46360345
		Actin 1	*A. pisum*	41785	5.3	136	53	gi|217330650
		Tropomyosin-2-isoform 1	*A. pisum*	32465	4.6	123	37	gi|193704626
		THAP domain-containing protein 1	*A. pisum*	23470	11	71	47	XP_008190184.1
		β-actin	*A. gossypii*	41816	5.3	123	45	gi|532165030
		Actin	*A. pisum*	22413	5	84	46	gi|169218603
		Formin-like protein 2	*Papilio machaon*	150188	9.5	75	16	KPJ08353.1
		Dystonin isoform X18	*A. pisum*	618797	5.9	78	10	XP_008184103.1
Environmental Information Processing								
		Calphotin	*A. pisum*	62983	8.9	144	35	gi|193706873
		Calphotin	*A. pisum*	72602	8.3	135	21	gi|193620175
		Store-operated calcium entry regulator STIMATE	*Frankliniella occidentalis*	25735	10.1	57	7	XP_026284975.1
		Elongation factor 1-alpha	*A. pisum*	40451	8.4	72	23	gi|641662724

	Ras-related protein Rab-24	*Aphidius gifuensis*	22869	6.2	62	23	XP_044010615.1
	Translation initiation factor eIF-2B	*A. pisum*	52730	10.2	60	15	XP_001950685.1
	14-3-3 protein zeta	*A. pisum*	28322	4.6	91	13	NP_001156510.1
Genetic information processing							
	GroEL	*B. aphidicola*	55489	5	120	25	AAR23319.1
	Symbionin symL	*A. pisum*	57989	4.9	195	44	gi|285430
	Proteasome subunit alpha type-1-like	*A. pisum*	30874	6.8	133	39	gi|193674111
	GroEL	*B. aphidicola*	55489	5	120	25	AAR23319.1
	GroEL	*B. aphidicola*	55869	4.9	70	14	AAO33049.1
	Ribosomal protein L13	*Culicoides sonorensis*	24642	11.4	51	16	gi|56199502
	RNA-binding protein FUS	*Drosophila bipectinata*	12892	10.1	59	21	XP_017095453.1
	Zinc finger protein 33B	*Metaseiulus occidentalis*	40072	6.2	58	10	gi|391333863
	Glycine--tRNA ligase subunit beta	*Rickettsia bellii*	46738	10.5	60	20	MCC8370817.1
Cellular process							
	Murein hydrolase activator EnvC	*R. insecticola*	29662	11	60	11	WP_006704068.1
	Vegetative cell wall protein gp1	*F. occidentalis*	39494	10	69	14	XP_026286246.1
Stress response							
	Cytochrome P450 6B5 isoform X1	*Bombus terrestris*	60378	9.3	64	10	XP_012168947.2
	Heat shock protein cognate 3 precursor	*A. pisum*	72993	5.1	74	11	gi|242397408
Others							
	1-acyl-sn-glycerol-3-phosphate acyltransferase	*Apis florea*	30660	10.1	75	26	gi|380023766
	Lipoyltransferase 1	*B. terrestris*	45791	9.5	66	15	gi|340712273
	Allatostatin A prohormone precursor	*Gryllus bimaculatus*	35149	9.6	65	17	CAC83078.1
	Spermidine synthase	*A. pisum*	33383	5.3	55	20	gi|242246955
	Hypothetical protein CWU_01330	*B. aphidicola*	6052	10.9	43	32	YP_005618305.1
	Hypothetical protein LOC100167736	*A. pisum*	72602	8.3	135	21	XP_001944564.1



Note: Mascot score, Mowse score according to Mascot search; MS coverage, percentage of the protein sequence identified; Protein. MW, molecular weight; PI, isoelectric point.

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Figure legends

Figure 1. A) Total number of aphids (mean ± SE) and B) weight reached (mean ± SE) by *S. avenae* individuals after 14 days of development on wheat and barley seedlings as a function of the presence or absence of the secondary endosymbiont *R. insecticola.* C) Root/shoot ratio of dry weight of wheat and barley plants after treated with *S. avenae* individuals depending on the presence or absence of the secondary endosymbiont *R. insecticola.*

Figure 2. Distribution of metabolic pathways for proteins with different expression levels in *S. avenae* depending on the occurrence of *R. insecticola* secondary symbiont on barley or wheat host plants.

621 **Figure 1**

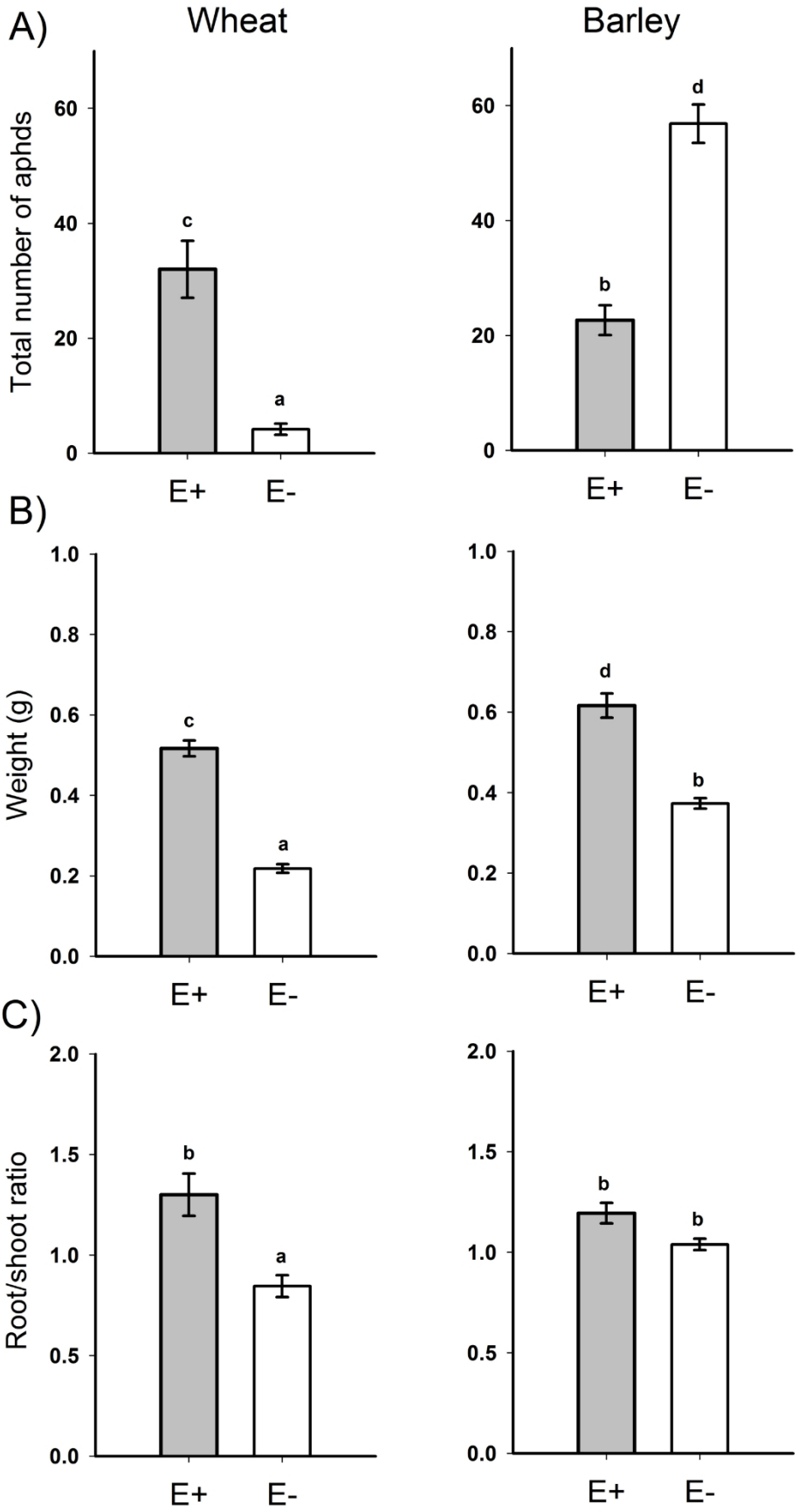

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Figure 2

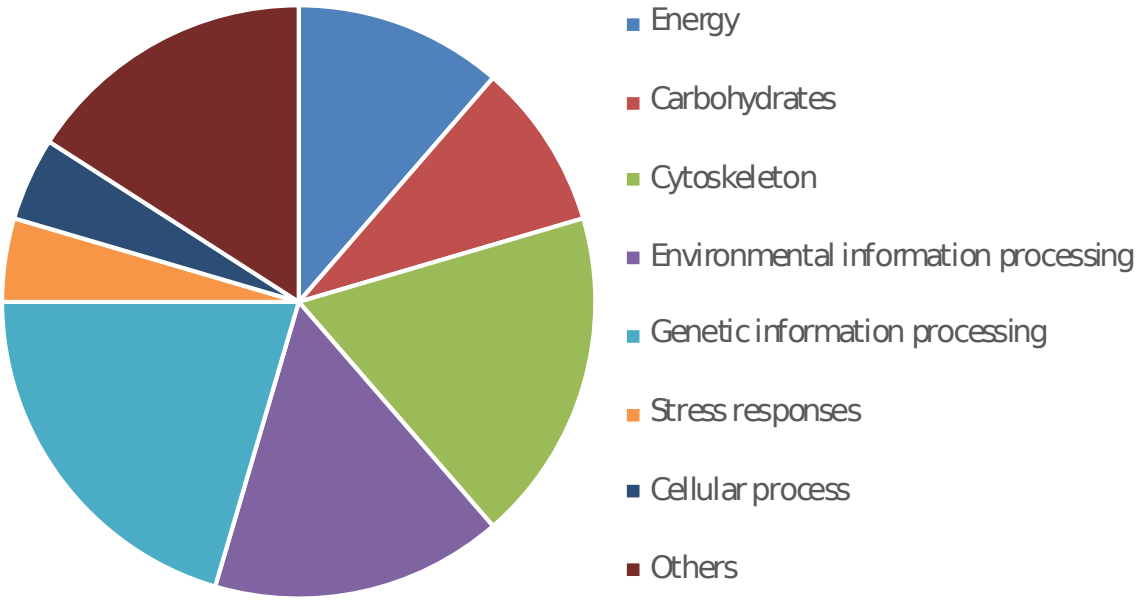
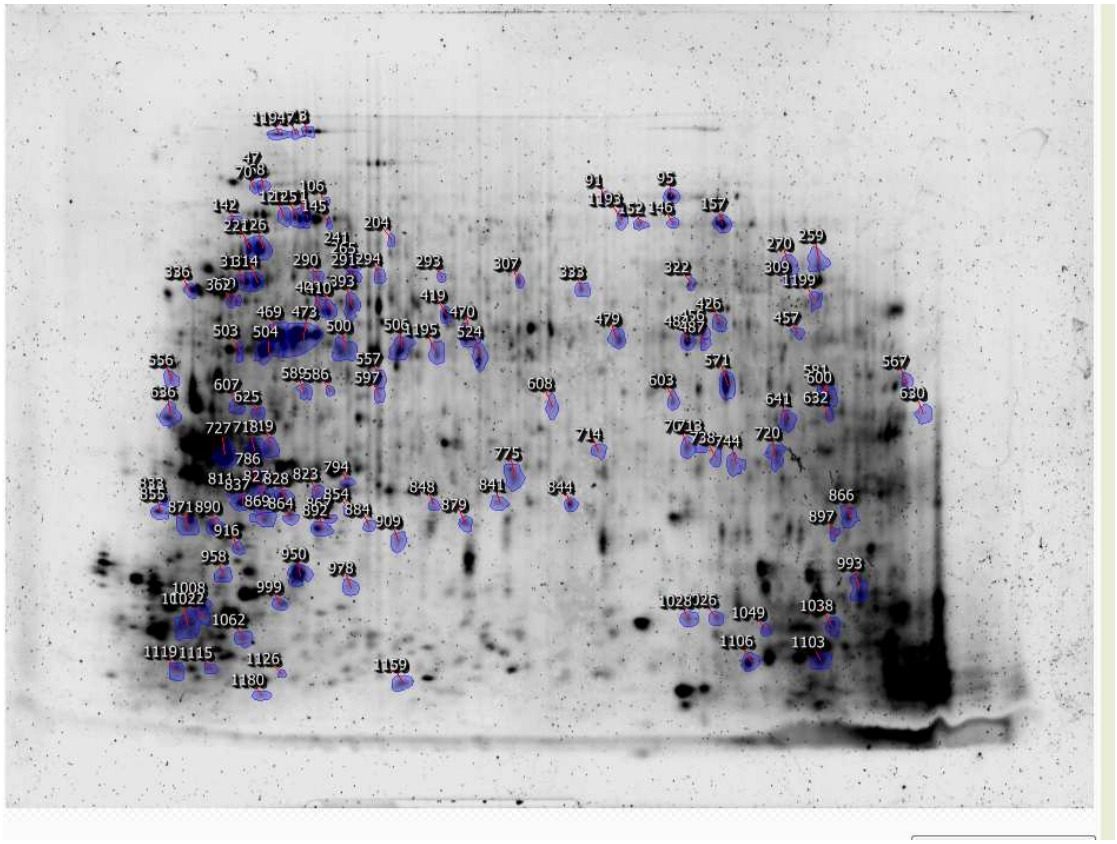
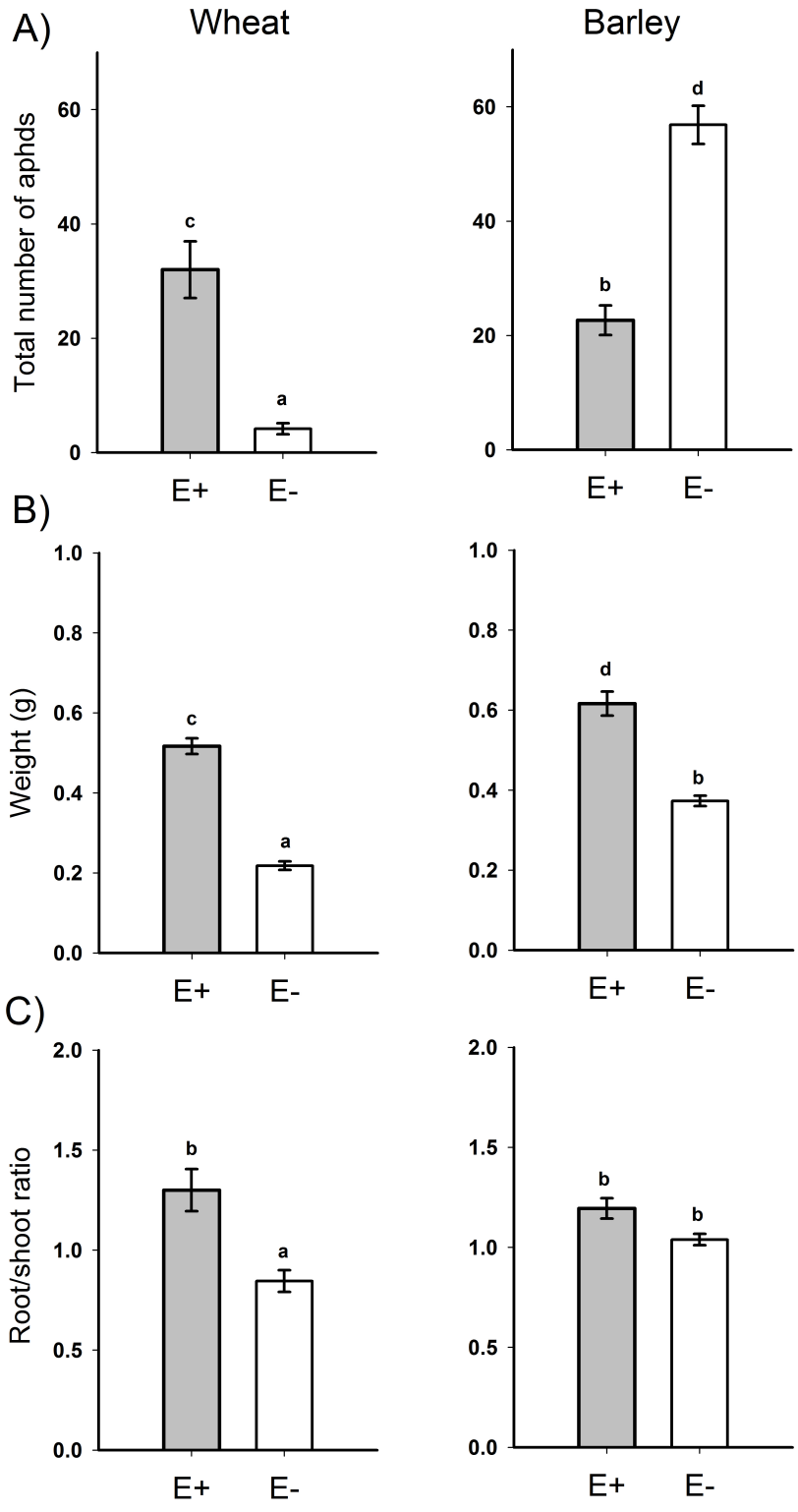

Energy
Carbohydrates
Cytoskeleton
Environmental information processing
Genetic information processing
Stress responses
Cellular process
Others

Figure S1.



Figures



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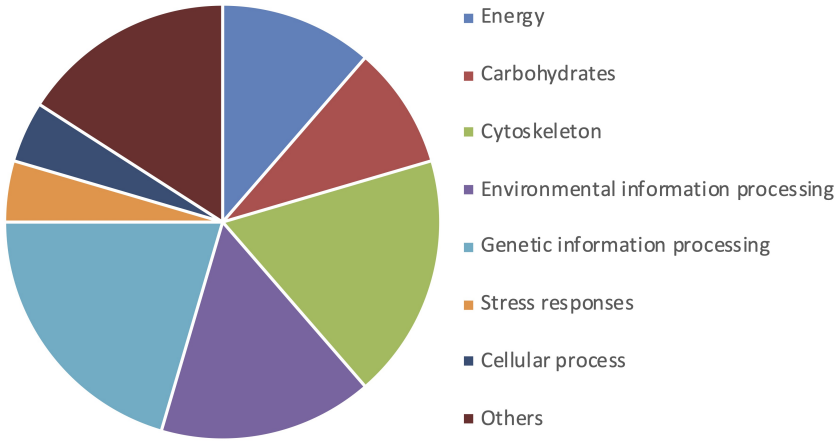

Figure 2

Figure 2. Distribution of metabolic pathways for proteins with different expression levels in *S. avenae* depending on the occurrence of *R. insecticola* secondary symbiont on barley or wheat host plants.