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Multiple infections influence the resistance of potato cultivars to late blight and potato cyst nematodes

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Abstract

Each season, crops face simultaneous or successive multiple infections. Yet cultivar resistance is often bred against one pest at a time, and screened under single infections. This raises the question of resistance expression bred under mono-infections when exposed to multiple infections. We tackled this question by measuring the level of quantitative resistance of potato cultivars to either *Globodera pallida* or *Phytophthora infestans* under single or multiple infections, using Potato virus Y (PVY) or *Rhizoctonia solani* as the co-infecter. Overall, co-infection resulted in less multiplication of the target pathogen compared to single infection, although this reduction did not always reach statistical significance. A preliminary assessment of defence gene expression in plants infected with *P. infestans* alone or together with PVY or *R. solani* suggested that *P. infestans* repressed defence genes previously triggered by PVY, but boosted the expression of these genes when primed by *R. solani*. Together, these data support the view that resistance expression is impacted by co-infection, and that it should be screened under multiple infection situations to be best assessed and exploited as a component of Integrated Pest Management strategies.

Introduction

Host resistance is critical for the efficient and sustainable control of many crop diseases. It is based on a range of physical and biochemical mechanisms, which allow to escape from infection, prevent the pathogen from entering host tissue, limit its spread within infected organs, and/or decrease secondary inoculum production and thus epidemic spread (Dangl & Jones, 2001). The genetics of these mechanisms (and of the pathogenicity determinants in the pathogens targeted) largely conditions their efficacy and durability (Jones & Dangl, 2006; Dangl *et al.*, 2013); they also allow several to be combined into a single host genotype, either by exploiting complementary or broad spectrum defence pathways (Glazebrook, 2005; Kissoudis *et al.*, 2014) or by targeting key genes with pleiotropic activity against multiple pathogens (McDowell & Woffenden, 2003).

Resistance breeding has been developed since the beginning of plant pathology, and is now used against a wide range of infectious diseases (Dangl *et al.*, 2013). However, most breeding programmes target one pathogen or disease at a time, whereas crop plants usually face sequential or simultaneous attacks by different pathogens or pests during a single season. This is particularly the case in vegetatively propagated crops, like potato, which are vulnerable to a wide range of pathogens and where both chronic and acute infections can occur (Rowe, 1993).

This raises the important question of the possible alteration (reduction or increase) of resistance bred under single infection conditions in a context of multiple infections. In potato, the available literature shows somewhat diverging results on disease severity in multiple vs. single infections. Indeed, Richardson & Doling (1957) showed an increased incidence and severity of late blight, caused by the oomycete *Phytophthora infestans*, on tubers of plants co-infected by Potato leaf roll virus (PLRV) compared to neighbouring virus-free plants. A similar observation was made in potato cultivars upon co-infection with Potato virus X (Dowley, 1973) or with Potato virus S (Lin *et al.*, 2014). However, Richardson & Doling (1957) also showed

that, under laboratory conditions, potato leaflets infected with PLRV are significantly less susceptible to infection by *P. infestans*, and that the expansion of late blight symptoms was also slower in virus-infected than in virus-free leaflets. They explained the higher apparent susceptibility of PLRV infected plants in the field as a side effect of the rolling of the leaves caused by infection, which helped retain moisture longer and hence favour infection by *P. infestans*, even though the leaf tissue itself was less susceptible to pathogen spread as shown by the laboratory tests, all performed under high humidity. These observations were later extended to other viruses and viroids (PVX, PVY, PLRV, PSTvd), under both field and controlled conditions (Fernandez de Cubillos & Thurston, 1975). Importantly, such a modulation of disease severity under co-infections is not restricted to aerial, polycyclic pathogens, but also occurs with soilborne, often monocyclic pathogens. For instance, Back *et al.* (2006) showed, in the field, that higher number of larvae of the potato cyst nematode *Globodera rostochiensis* resulted in more severe symptoms of black scurf (*Rhizoctonia solani*). Moreover, above-ground infections could affect below-ground pathogens and *vice versa*. Hoysted *et al.* (2018) showed for instance that root exudates produced by potatoes infested by the aphid *Myzus persicae* induced significantly lower hatching of the cyst nematode *Globodera pallida* than those from un-infested control plants.

While these data suggest that co-infection can strongly affect disease susceptibility, most of the aforementioned results were obtained on cultivars susceptible or highly susceptible to the corresponding disease. We have found little information as to the behaviour of cultivars with quantitative (partial) resistance under co-infection. This issue is however critical for the sustainable deployment and optimal use of these valuable resistant resources. Furthermore, there is even less information about the resistance molecular pathways that could be altered during co-infection compared to single infections, although Petek *et al.* (2014) showed that

infection by PVY repressed plant defence pathways active against Colorado potato beetle (*Leptinotarsa decemlineata*).

The aim of this study was therefore to investigate the effect of co-infections on the performance of quantitative plant resistance bred under mono-infections against two major potato pathogens (one telluric and monocyclic, the other aerial and polycyclic in nature), and to start exploring their impact on major defence pathways expressed in potato. To this end, we used potato cultivars showing high levels of quantitative resistance to the late blight pathogen *P. infestans* or the cyst nematode *G. pallida*. The pathogens used as co-infectors were *R. solani* and potato virus Y (PVY). The four combinations tested therefore correspond to three types of pathogen pairs: either two monocyclic soil-borne pathogens (*G. pallida* + *R. solani*), a monocyclic telluric and a polycyclic aerial (*G. pallida* + PVY, *P. infestans* + *R. solani*) or two aerial polycyclic pathogens (PVY + *P. infestans*); the pathogens also belong to different taxonomic Reigns – Fungi, nematodes (Animalia), oomycetes (Chromista) and viruses (Viria). Therefore, the combinations tested were designed to be ‘model systems’ covering most possible epidemic situations, and thus allowing inference of some generic observations and conclusions.

Materials and methods

Partially resistant cultivars for co-infection experiments

The potato cultivar Iledher was selected to test the effect of co-infections on the efficiency of quantitative resistance to the potato cyst nematode *Globodera pallida*. Iledher was the first potato cultivar resistant to *G. pallida* registered in the French catalogue. Its resistance is derived from *Solanum vernei*, and depends on the QTL *GpaV_{vm}*, which maps on potato chromosome V and explains a large part of the genetic variance for resistance to *G. pallida* in segregating progenies (Roupe van der Voort *et al.*, 1998, 2000). Currently, all European potato cultivars resistant to *G. pallida* have *S. vernei* in their genealogy, and bear *GpaV_{vm}*. This QTL acts by

masculinizing nematode populations: it is responsible for the development of most nematodes into adult males (Fournet *et al.*, 2013).

The potato cultivar Robijn was chosen to explore the effect of co-infections on the efficiency of quantitative resistance to the potato late blight pathogen *P. infestans*. Robijn is an old Dutch cultivar (first registered in 1926) and is a suitable standard for lasting and spatially stable quantitative resistance to late blight (Dowley *et al.*, 1999; Forbes *et al.*, 2005). The partial resistance of cv. Robijn acts notably by reducing spore production of *P. infestans*, and to a lesser extent by lengthening the latent period and decreasing the daily lesion growth rate (Clément *et al.*, 2010).

Neither Iledher nor Robijn have reported genes for resistance to *Rhizoctonia solani* or PVY.

Pathogen strains and/or populations for co-infection experiments

The *G. pallida* cysts used in this study came from the population Ouessant (called FR-1 by Plantard *et al.*, 2008). Cysts used as inoculum for experiments 1 and 2 were produced on the susceptible potato cultivar Désirée in 2012 and 2013, respectively. Cysts were calibrated between 350 and 500 μm , and the number of juveniles per cyst was estimated for each inoculum.

The selected *P. infestans* isolate (13P.19.03) belongs to the EU_6_A1 multilocus lineage. Clonal lineage EU_6_A1 is one of the most aggressive in current European populations (Mariette *et al.*, 2016). Isolate 13P.19.03 was isolated from potato cultivar Bintje in 2013, and kept in the INRAE IGEPP working collection as axenic cultures on pea agar.

A strain of *Rhizoctonia solani* anastomosis group 3 (AG3) was selected for the experiments. AG3 is predominantly associated with Solanaceous crops, and is divided into three genetically different subgroups: AG3PT associated with potato, AG3TB associated with

tobacco (Kuninaga *et al.*, 2000; Woodhall *et al.*, 2008) and AG3TM associated with tomato (Bartz *et al.*, 2010). We used the AG3PT strain denominated MIAE261 and isolated by Fiers *et al.* (2011).

The PVY isolate PVY^N605 was used for the corresponding co-infections. This isolate is the reference of the PVY^N group (Jakab *et al.*, 1997), and induces mild symptoms of mosaic on most potato cultivars with no resistance genes. This isolate was maintained on tobacco plants in greenhouse conditions before the beginning of the experiments.

Co-infection protocols

Globodera pallida experiments. The trial included three conditions: the first corresponded to plants inoculated with *G. pallida* only (Ø-Gp), the second to plants co-inoculated with *G. pallida* and *R. solani* (Rhizo-Gp), and the third to plants co-inoculated with *G. pallida* and PVY (PVY-Gp). Two independent replicate experiments were performed (experiment 1 in 2015 and experiment 2 in 2016), with twelve replicate plants in each condition and each year.

To perform *G. pallida* inoculation, 20 cysts were placed in a tulle bag deposited in a 13-cm pot, three-quarter filled with a soil mixture (2/3 sand and 1/3 natural field soil) free of cysts. One tuber of Iledher was then planted in each pot, and covered with the same soil mixture. Potatoes were grown in the greenhouse, under natural light and regulated temperature (15°C for 8h and 20°C for 16h), for 120 days. This duration allowed newly formed cyst to become brown and detach easily from decaying root tissue, ensuring optimal recovery of these cysts from soil and accurate counting; it allowed only one generation of the nematode. Plants were watered with a nutrient solution (Hakaphos; NPK 15/10/15) twice during the experiment.

Rhizoctonia solani was inoculated onto plants of the Rhizo-Gp modality directly at planting. The inoculation consisted in depositing a piece of agar (4 mm diameter), cut from the actively growing margin of a pure axenic culture grown for four days on malt-agar in the dark,

next to a potato sprout on the tuber skin. Each inoculated tuber was then covered with a 10 cm layer of the potting mixture used for growing the plants. *R. solani* infection was checked at the end of the experiment on daughter tubers using a 0 – 5 visual scale (0 = no symptom ; 1= 0-5%, 2 = 6–25%; 3 = 26–50%; 4 = 51–75%; 5, > 75% of tuber or stem area covered by sclerotia or necrosis).

PVY inoculation was carried out on the plants of the PVY-Gp modality following the standard mechanical inoculation procedure described by Lacroix *et al.* (2010). Briefly, 1 g of infected tobacco leaf tissue was ground in the presence of 4 mL of inoculation buffer (50 mM Na₂HPO₄·12H₂O; 50 mM KH₃PO₄; 40 mM sodium diethyldithiocarbamate, pH 7.2) supplemented with 1.5 g of activated charcoal and 2.5 g of carborundum. This mixture was rubbed with fingers on two leaves of potatoes at the 4-leaf stage, three weeks after planting. Forty days after inoculation, successful PVY infection was checked using the RT-qPCR procedure described by Kogovšek *et al.* (2008).

Phytophthora infestans experiments. As for *G. pallida*, the trial included three conditions: the first corresponded to healthy plants (Ø-Pi), the second to plants inoculated with *R. solani* (Rhizo-Pi), and the third to plants inoculated with PVY (PVY-Pi) before inoculation with *P. infestans*. Two independent replicated experiments were performed (experiment 1 in 2016 and experiment 2 in 2017). In experiment 1, 12 plants were used per modality, and two leaflets per plant were inoculated with *P. infestans* (*i.e.* 24 replicates). In experiment 2, 20 plants were used per modality, and two leaflets per plant were inoculated (*i.e.* 40 replicates).

Plants were grown in a glasshouse regulated at 15-20 °C (night/day temperatures) and 16 h of light, in 13 cm pots (one tuber per pot) filled with a sand-peat-compost mixture. Plants were watered with a nutrient solution (Hakaphos; NPK 15/10/15) once a week. *R. solani* and PVY were inoculated as previously described.

To restore pathogenicity possibly lost during axenic cultures, the selected *P. infestans* isolate was first multiplied on detached leaflets of the susceptible cultivar Bintje (Mariette *et al.*, 2018). Sporangia produced on Bintje leaflets were collected in sterile water, and the concentrations of the resulting suspensions were adjusted to 5×10^4 spores.mL⁻¹. Suspensions were kept at 4 °C for approximately 2-3 h to promote zoospore release. Leaflets, collected from 6-8 week-old Robjin plants, were placed abaxial face up on the lids of inverted Petri dishes containing 10 g.L⁻¹ water agar, and infected by depositing a 20 µL drop of the calibrated sporangia suspension at the leaflet centre.

Resistance scoring

Globodera pallida experiments. After 120 days, newly formed cysts were extracted from the soil, using a Kort elutriator. The number of cysts was counted under a magnifying stereomicroscope. To evaluate the number of progeny per cyst, ten cysts were randomly picked from each replicate, pooled, and crushed in water before the eggs and juveniles were counted twice under a magnifying stereomicroscope.

Phytophthora infestans experiments. Infected leaflets were incubated for five days under controlled conditions (15 °C/18 °C night/day temperatures, 18 h daylight). Lesion diameters were measured in two perpendicular directions and lesion size was computed assuming an elliptic shape. Each leaflet was then washed in 10 mL saline buffer (Isoton II) and the number of spores was counted with either a Beckman Coulter Z2 (Beckman, Villepinte, France) or an Occhio Flowcell FS200 (Occhio France, Bailleau le Pin, France) cell counter.

Transcriptomic analysis of key defence gene expression

An exploratory transcriptomic analysis of the expression kinetics of 12 key defence genes, known to be involved in potato defence pathways against pathogens (Table 1) and included in

the qPFD potato chip (INRA patent: WO/2011/161388), was conducted on a set of leaflets of cv. Robijn prepared at the same time as those used for the pathogenicity measurements and collected 4, 5 and 6 days after inoculation with *P. infestans* during the second experiment with *P. infestans*. All protocols used were previously developed and validated (Thomas *et al.*, 2019).

For each condition (\emptyset -Pi, Rhizo-Pi and PVY-Pi), a single, bulk sample of 12 leaflets collected from different plants was harvested, immediately deep-frozen in liquid nitrogen, and lyophilized. The infection status of each individual plant at the time of sample collection was not known and could not be reliably ascertained (too early stage for *R. solani* infection for instance); therefore, potential differences in gene expression between plants might not have been usefully interpreted in terms of disease development, prompting us to favour bulk samples. After grinding, two 15 mg aliquots per sample were subsequently used for defence gene analysis. Extraction of mRNA from each aliquot was performed using the "SV Total Isolation System" kit of Promega. The integrity and quality of the extracted mRNA was checked using a Nanodrop 2000 (Thermo Scientific) and through electrophoresis on a 1 % agarose gel. cDNA was synthesised in a RT-PCR using Biorad's "iScript cDNA Synthesis" kit. The quality of cDNA was checked through electrophoresis on 1.5 % agarose gels. For each RT-PCR reaction, transcript accumulation values were calculated by normalizing the CTs obtained for each gene using the average CT of the reference housekeeping gene (ribosomal protein *L2*). The whole experimental process (extractions and RT-PCR amplification) was performed independently three times for each aliquot, yielding a total of six technical replicates (2 aliquots * 3 extractions/amplifications) per sample.

Data analysis

All statistical analyses were carried out using the appropriate packages of the statistical software R, version 4.1.1 (R Core Team, 2021).

Resistance data.

Normality and homogeneity of variances of residuals were checked with the Shapiro-Wilk and the Levene tests, respectively. Regarding the resistance of Iledher, the effects of modality (\emptyset -Gp, Rhizo-Gp and PVY-Gp), experiment (1 and 2) and the corresponding interaction on the number of cysts and the number of juveniles per cyst were tested through a F-test on a linear model (function 'lm'). Regarding the resistance of Robjin, the effects of modality (\emptyset -Pi, Rhizo-Pi and PVY-Pi), experiment (1 and 2) and the corresponding interaction on the lesion size and the number of spores were tested through a Wald chi-square test on a linear mixed model (function 'lmer') considering plant as a random factor (because two leaflets were collected by plant). Because of significant interaction effects in both trials, analyses were performed separately for each independent experiment, using a linear model for Iledher and a linear mixed model for Robjin (with plant as a random factor). When significant effects were detected, pairwise comparisons of mean values were computed using an analysis of contrasts (function 'emmeans', $\alpha = 0.05$).

Transcriptomic data. After checking the normality and homogeneity of variances of residuals, transcript accumulation data were submitted to a linear mixed model (function 'lmer') considering aliquot as a random factor (because three extractions and PCRs were performed for each aliquot). When significant effects were detected, pairwise comparisons of mean values were computed using an analysis of contrasts (function 'emmeans', $\alpha = 0.05$).

Results

Effect of co-infections on resistance and pathogen life history traits

Globodera pallida. Co-infection conditions effects were significant on the number of *G. pallida* cysts produced on Iledher and on juvenile numbers per cyst (Table 2). The experiment effect was significant for cyst contents, but not for cyst numbers. The interaction between those factors was significant and large enough to change the ranking of the three co-infection conditions

between experiments (Table 2). In the first experiment, the number of cysts was significantly reduced by co-infection with either *R. solani* or PVY, ($F_{2,33} = 11.51$ and $P = 0.0002$; Figure 1), as were juvenile numbers per cyst ($F_{2,32} = 10.64$ and $P = 0.0003$; Figure 1). In the second experiment, there were no significant differences between conditions for either cyst numbers ($F_{2,33} = 0.19$ and $P = 0.830$; Figure 1) or cyst contents ($F_{2,19} = 1.77$ and $P = 0.198$; Figure 1), despite a trend towards fewer cysts and offspring produced in the Rhizo-Gp modality. The comparison between both experiments showed that under the experimental conditions of the first year, the number of cysts produced was higher but that these cysts contained fewer juveniles than under the experimental conditions of the second year (Figure 1).

Phytophthora infestans. Co-infection condition and experiment effects were highly significant on both lesion size and spore numbers produced by *P. infestans* on potato cultivar Robijn (Table 3). In the first experiment, neither lesion sizes ($\chi^2 = 2.94$, $df = 2$ and $P = 0.229$) nor spore numbers ($\chi^2 = 5.57$, $df = 2$ and $P = 0.056$) significantly differed between conditions, although they tended to be lower on leaflets from plants infected by *R. solani* or PVY than on control leaflets (Figure 2). In the second experiment, lesions were significantly smaller under co-infection of *P. infestans* with PVY compared to co-infection with *R. solani* or infections with *P. infestans* only ($\chi^2 = 23.72$, $df = 2$ and $P > 0.0001$; Figure 2). The number of spores was lower on co-infected than on mono-infected leaflets ($\chi^2 = 22.94$, $df = 2$ and $P > 0.0001$; Figure 2). The comparison between both experiments showed that under the experimental conditions of the first year, lesions were smaller and produced fewer spores than under the experimental conditions of the second year (Figure 2).

Defence pattern alteration during co-infection

In these preliminary experiments, the various inoculation conditions elicited changes in the expression of key defence genes in cv. Robijn. Six days after the second inoculation in co-infection modalities, the expression of most genes increased in plants inoculated with PVY alone (*PR4*, *PAL*, *WRKY1*, *EIN3* and *Cals2*), and for some genes with *R. solani* alone (*THT* and *EIN3*). Infection by *R. solani* and PVY generally triggered the expression of defence genes belonging to the salicylic acid (*PAL*, *THT*, *WRKY1*) and the jasmonic/ethylene acid pathways (*PR4* and *EIN3*). By contrast, *P. infestans* significantly repressed the expression of these genes, a sign of active counter-defence. Overall, transcript accumulation decreased for most genes, with only few exceptions (*PRI* and *CAT*), in leaflets inoculated with *P. infestans*, compared to the corresponding non-inoculated control (Figure 3). Similar trends were observed in samples collected four and five days post inoculation (data not shown).

Moreover, the expression of defence genes in situations of double infections was also affected, compared to both control and singly-infected plants, but the direction of the change depended on both the initial infector and the nature of the genes. For instance, the addition of *P. infestans* to plants previously infected with *R. solani* boosted the expression of *PR* genes, whilst infection with *P. infestans* of plants previously infected with PVY tended to decrease the expression of these genes (Figure 3). Overall, the results provided indications that infection by *P. infestans* tended to decrease the expression of defence genes initially triggered by PVY, but boosted that of genes primed by *R. solani* (*PRI-4*, *CAT*, *POX*, *EIN3*).

Discussion

The overall goal of this study was to assess the performance of resistance bred under mono-infections under conditions of multi-infections, and to explore some of the physiological mechanisms underlying this performance. As plant resistance is gaining major importance in integrated pest management schemes, and since these schemes should target not only a single

pest but crop health at large, it becomes critical to determine whether multi-infections could improve, or alter, resistance levels compared to the standard assessments, usually made with only one disease at a time.

Our data showed that co-infections reduced the quantity of symptoms and/or pathogen reproduction of both *G. pallida* and *P. infestans* in partially resistant potato cultivars, irrespective of whether the co-infecter was PVY or *R. solani*. While the magnitude of this reduction varied according to pathosystems and experiments, the trend towards resistance enhancement was consistent across all experiments: over the eight observations (2 experiments * 2 co-infection conditions * 2 variables measured) made for each pathogen (*P. infestans* and *G. pallida*), eight and seven, respectively, showed a lower level of resistance in the mono-infections than in the co-infection conditions. Such frequencies are extremely unlikely to occur by chance only ($P = 0.0039$ for *P. infestans*; $P = 0.0078$ for *G. pallida*). This supports the conclusion that co-infections consistently enhance resistance levels in these pathosystems, as do observations of late blight development on a small number of Robijn plants grown in pots under natural, outdoor conditions, which also showed that co-infection with *R. solani* reduced late blight severity (data not shown). These results extend earlier observations made on susceptible cultivars (e.g. Fernandez de Cubillos & Thurston, 1975) to hosts with high levels of partial resistance.

While co-infections consistently tended to increase resistance levels, the magnitude of their effect was generally moderate, and sometimes failed to reach statistical significance. This is quite usual when working with resistant genotypes, which lower the means and variances of measured traits. However, it is important to recall that apparently small differences in life history traits can have deep evolutionary or demographic consequences. This is particularly true with polycyclic pathogens, as is the case of *P. infestans*: our experiments were made on a single pathogen multiplication cycle, whereas in real-time epidemics, the differences noted at each

cycle will increase in a multiplicative way. As outline above, we have good reasons to believe that the differences in resistance expression we measured between singly and multiply-infected plants are correctly attributed to the effect of co-infections and not to chance variability, and therefore constitute an important biological result.

Differences in the magnitude of co-infection effects between replicate experiments might result from experimental differences in the intensity of infection and/or of ecological interactions. For instance, the effect of co-infections on the resistance to *G. pallida* of the potato cultivar Iledher was strong and significant in experiment 1, but non-significant in experiment 2. Counting the number of juveniles per cysts, using three additional bags of 20 cysts, suggested that competition was much more intense in experiment 2 (with 379 juveniles per cyst on average) than in experiment 1 (165 juveniles per cyst on average). This increased competition in experiment 2 could have led to the production of more males and fewer females (Ross & Trudgill, 1969; Triantaphyllou, 1973). To confirm this hypothesis, a similar experiment would need to be done using graded cyst numbers or pre-hatched infective juveniles as inoculum, from a low to a strong competition between juveniles. Such variations make the design and implementation of robust co-inoculations protocols really challenging, especially when using soilborne organisms and long growing periods.

A shared feature of the resistances evaluated here was to act directly on pathogen reproduction, as both the resistance of Iledher to *G. pallida* and of Robijn to *P. infestans* primarily reduce pest reproduction compared to that allowed by susceptible cultivars (Clément *et al.*, 2010; Fournet *et al.*, 2013). One could thus wonder whether the resistance increase observed under co-infections applies to all types of resistance, or only to those limiting pathogen reproduction. Our data do not provide an answer to this question. However, they echo recent observations made in another pathosystem, the *Ascochyta* blight complex of peas, where

co-infection by different species of the same complex also reduced the number of infectious offspring compared to single infections (Dutt *et al.*, 2021).

Contrary to our initial hypothesis, the reduction in late blight symptoms upon co-infection with *R. solani* or PVY could not be related to a sharp increase in defence gene transcripts. The quantification experiments provided preliminary evidence suggesting that *P. infestans* develops a strong counter defence activity against a wide range of defence pathways. This is consistent with earlier observations (Thomas *et al.*, 2019; Martin *et al.*, 2020) and supports the idea that *P. infestans* fitness is directly linked to its ability to maintain a biotrophic interaction with its host for as long as possible (Kröner *et al.*, 2019). Therefore, it can be hypothesised that the necessity for the pathogen to counter a wide array of defence mechanisms mobilises a large bulk of its metabolic resources, that it cannot direct to foster its growth – and hence the exploitation of its host and the production of the resulting symptoms. This would therefore result in ‘resistance by exhaustion’, led by a sharp change in resource allocation increased by co-infection. A similar output, but for a different reason, might be involved in the fitness reduction of *G. pallida* under co infections. Indeed, the nematode fitness is strongly conditioned by nutrient availability (Bell *et al.*, 2022), that co-infection mechanically decreases due to competition for the same resources. These hypotheses need to be confirmed by more complete experiments, but open interesting perspectives for the use of biocontrol products (in particular plant defence stimulators) and multiple resistance in integrated control strategies. If validated, they would support the idea to combine multiple resistance pathways, without searching for maximal efficacy of each, rather than single, highly efficient mechanisms that would divert only one kind of resources for the pathogen.

The role of defence mechanisms and pathways in multiple infections of partially resistant plants has been so far little explored. Our data suggest a complex pattern of interactions, involving both defence induction, defence priming and counter-defence according

to the co-infecting pathogens. Given the known antagonism between jasmonic acid- and salicylic acid- mediated pathways and their differential involvement in response against either biotrophs or necrotrophs (Glazebrook, 2005; Pieterse *et al.*, 2009, 2012), it is not surprising that such an array of interaction occurs during co-infections. While our data provide a first glimpse at such arrays, a lot remains to be done to fully explore their kinetics, intensity and final impact on disease severity.

This work provides some new insights into the effect of multiple infections on the expression of host resistance. By showing that the efficacy of resistances bred under single infections changes in different co-infection contexts, it questions the need to assess host resistance not only with one, but with several interacting pathogens. This will be critical to best exploit such resistances in crop protection systems shifting the current paradigm (managing single diseases separately) to a holistic control of plant health.

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Data accessibility

The raw data files corresponding to the *G. pallida*, *P. infestans* and defence transcript accumulation experiments are deposited on the IGEPP dataverse of the data.inrae.fr portal. The

corresponding DOI are 10.15454/D9IJWR, 10.15454/C4QOET and 10.15454/QH1MV7. They will be made publicly available upon acceptance of the manuscript.

Accepted Article

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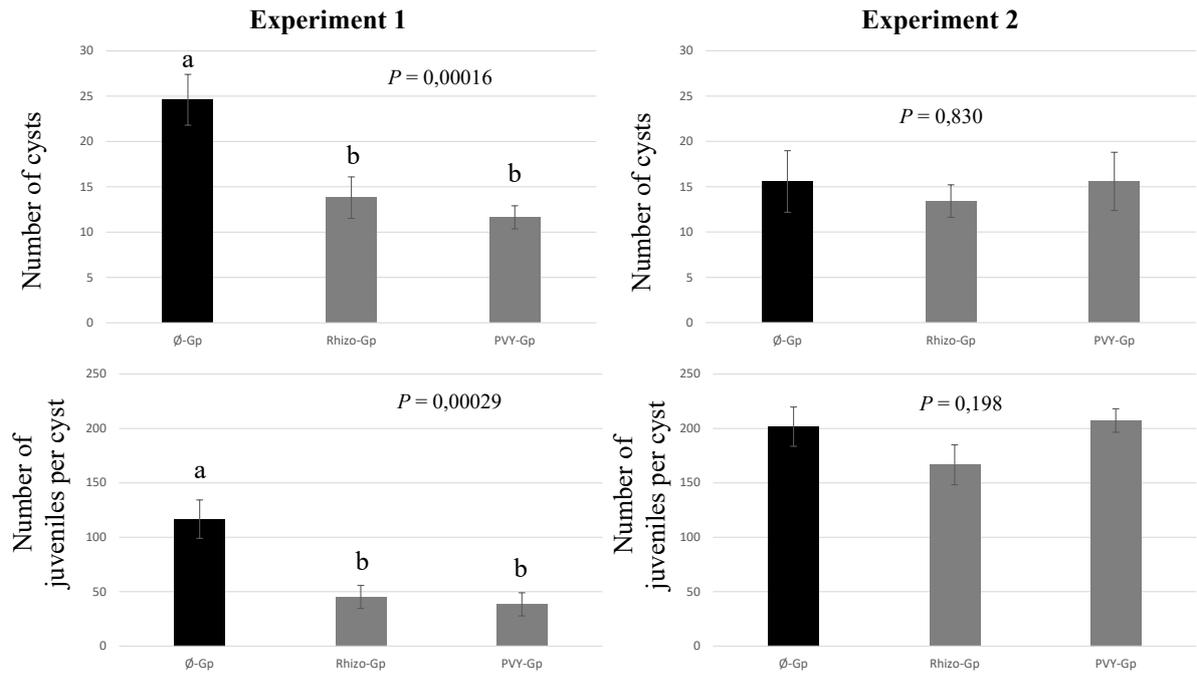
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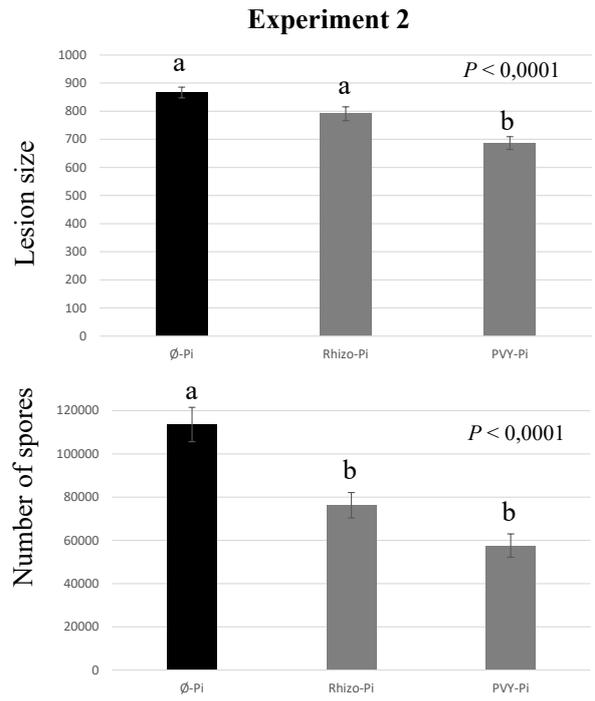
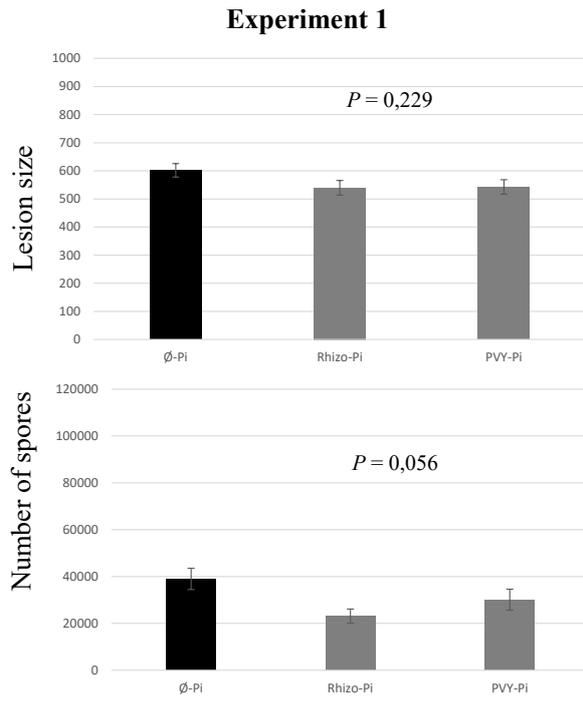
Figure legends

Figure 1. Number of cysts and number of juveniles per cyst (means \pm SEM) produced on potato cultivar Iledher inoculated by *Globodera pallida* only (\emptyset -Gp), co-inoculated with *G. pallida* and *Rhizoctonia solani* (Rhizo-Gp), or co-inoculated with *G. pallida* and potato virus Y (PVY-Gp). Results of the experiments 1 and 2 were shown by graphs on the left and on the right, respectively.

Figure 2. Lesion size and number of spores (means \pm SEM) produced on potato cultivar Robjin inoculated with *Phytophthora infestans* only (\emptyset -Pi), co-inoculated with *P. infestans* and *Rhizoctonia solani* (Rhizo-Pi), or co-inoculated with *P. infestans* and the potato virus Y (PVY-Pi). Results of the experiments 1 and 2 were shown by graphs on the left and on the right, respectively.

Figure 3. Expression levels (means + SEM) of selected defence genes in leaflets from potato plants non-infected (water), infected with *Rhizoctonia solani* (Rs), PVY or *Phytophthora infestans* (Pi) in single or double infections. Transcript accumulation levels were measured six days after the second inoculation, and normalised relative to that of a reference housekeeping gene (ribosomal protein L2) in the same PCR reactions.





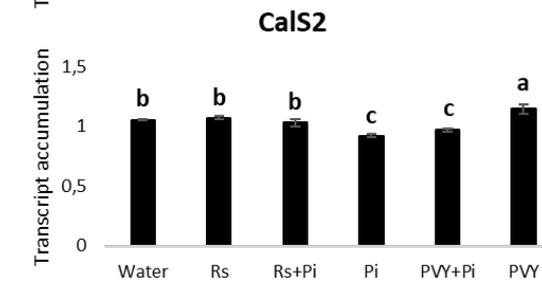
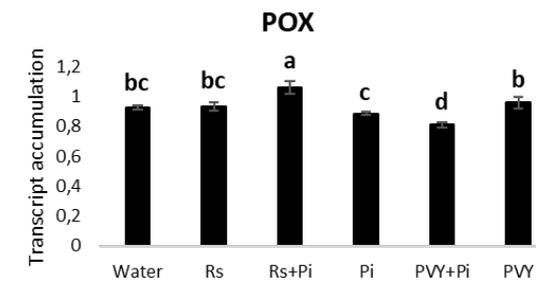
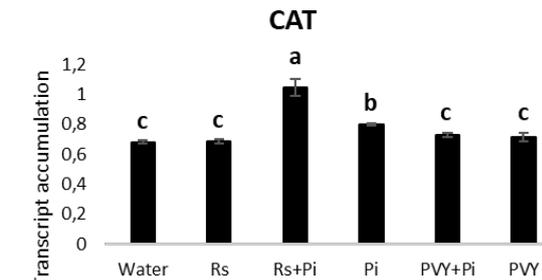
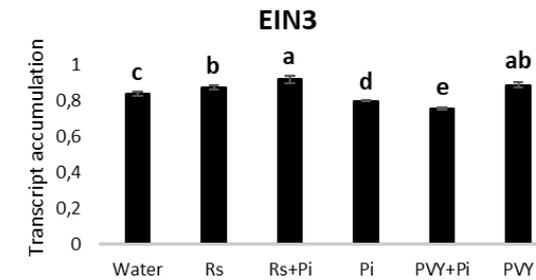
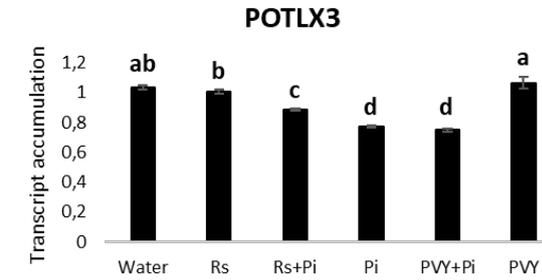
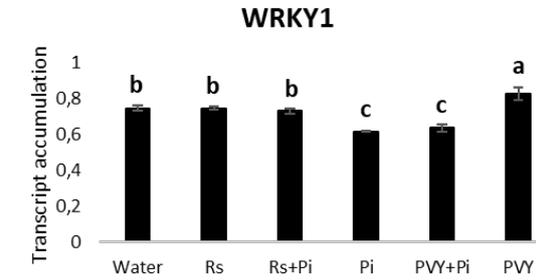
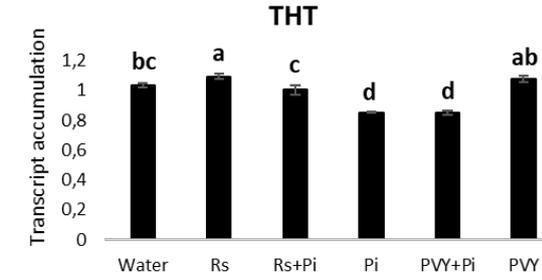
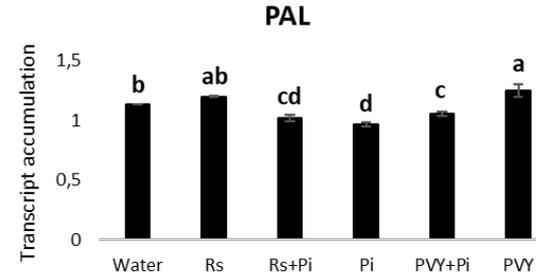
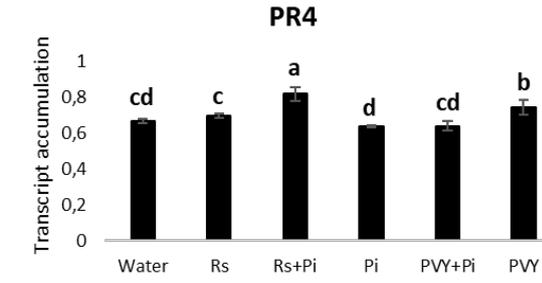
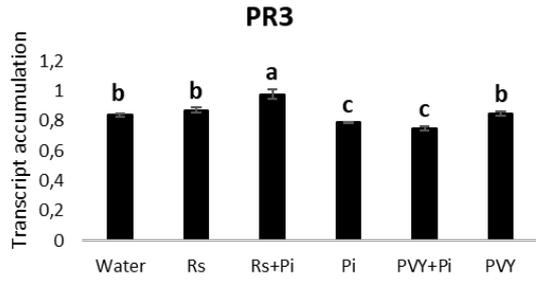
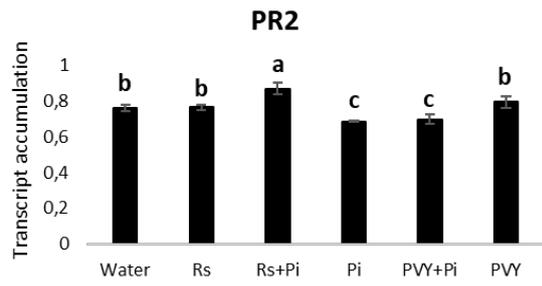
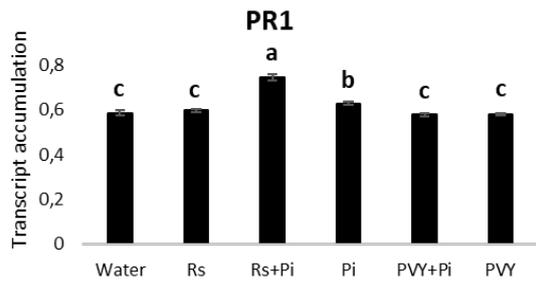


Table 1. List of defence and control housekeeping (ribosomal protein *L2*) genes used for the transcriptomic analysis of defence reactions under single and multiple infections.

Defence pathway	Gene Name	Gene function	GenBank n°
PR proteins	<i>PR1</i>	Pathogenesis-related protein 1	AJ250136
	<i>PR2</i>	Pathogenesis-related protein 2 (β -glucanase)	U01901
	<i>PR3</i>	Pathogenesis-related protein 3 (chitinase)	AF024537
	<i>PR4</i>	Pathogenesis-related protein 4 (hevein-like)	AM908515.1
Phenylpropanoid pathway	<i>PAL</i>	Phenylalanine ammonia-lyase 1	X63103
	<i>THT</i>	Potato tyramine N-(hydroxycinnamoyl) transferase	AB061243
Transcription factor of SA pathway	<i>WRKY1</i>	WRKY transcription factor 1	AJ278507
JA pathway	<i>POTLX3</i>	Potato locus lipoxygenase	U60202
Et pathway	<i>EIN3</i>	EIN3-BINDING F BOX PROTEIN 1	CV082047
Parietal modification	<i>CAT</i>	Catalase	Z37106
	<i>Cals2</i>	Callose synthase	CN496203
Antioxidant system	<i>POX</i>	Potato peroxidase (putative peroxidase)	AM231411
Reference housekeeping gene	<i>L2</i>	Potato ribosomal protein L2	CK259681.1

Table 2. Effects of modality (Ø-Gp, Rhizo-Gp and PVY-Gp), experiment (1 and 2), and the corresponding two-way interaction on the number of cysts and on the number of juveniles per cyst produced by *Globodera pallida* on the resistant potato cultivar Hedher (F-test on a linear model).

Source of variation	df	Number of cysts		Number of juveniles per cyst		
		F-value	P-value	df	F-value	P-value
modality	2	4.15	0.020	2	8.15	0.0008
experiment	1	0.44	0.507	1	109.60	<0.0001
modality:experiment	2	3.52	0.035	2	4.21	0.020
error	66			51		

Table 3. Effects of modality (Ø-Pi, Rhizo-Pi and PVY-Pi), experiment (1 and 2), and the corresponding two-way interaction of these factors on the level of resistance, *i.e.* on the lesion size and on the number of spores of *Phytophthora infestans*, of the potato cultivar Robjin (Wald test on a linear mixed model with plant as a random factor).

Source of variation	df	Lesion size		df	Number of spores	
		Chisq	<i>P</i> -value		Chisq	<i>P</i> -value
modality	2	23.47	<0.0001	2	25.83	<0.0001
experiment	1	88.08	<0.0001	1	60.58	<0.0001
modality:experiment	2	5.37	0.068	2	8.39	0.015