Host nutrient supply mediates facilitation and competition between plant viruses

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Abstract

Ecological theory that predicts the effects of resource availability on species interactions has been explored across a range of systems. Yet, application of this theory to communities of host-associated pathogens has been limited. Host resources and diet can impact disease severity and prevalence, and these resource effects on disease may be mediated by pathogen-pathogen interactions within hosts infected with multiple pathogens. As with free-living organisms, pathogen species can alter each other's population growth rates, population densities, and transmission to new hosts through facilitative or antagonistic processes. We used a model grass-virus system (Barley and Cereal Yellow Dwarf Viruses) to test the hypothesis that host nutrition can constrain virus-virus interactions by simultaneously determining both within-host density and transmission to new hosts. Hosts (oats) were grown in growth chambers with different concentrations of soil nitrogen (N) and phosphorus (P) and infected with one or both viruses (i.e.,

coinfection). We quantified the impacts of nutrient addition on virus-virus interactions through within-host density (i.e., the concentration of viruses in a plant) and transmission to new hosts. Nutrients promoted facilitation of one virus (CYDV-RPV) through increased density (elevated N) and increased transmission (elevated N and P) with coinfection relative to single infection. The other virus (BYDV-PAV) experienced facilitation through increased density when nutrients were limited, but nutrient addition led to antagonistic effects of coinfection on density (elevated N and P) and transmission (elevated N). Our results highlight opportunities for novel insights from testing predictions of community ecology in disease systems, including nutrient-dependent facilitation and nutrient-mediated interactions during transmission that were not predicted by within-host dynamics. This study contributes to the growing literature on ecological interactions among coinfecting pathogens by demonstrating that resource availability can mediate pathogen-pathogen interactions both within hosts and during transmission.

Key words: coinfection, facilitation, resource competition, transmission, within-host, barley and cereal yellow dwarf viruses

Introduction

Resource supply rates can alter the outcome of many species interactions, including competition (Tilman 1977), predation (Oksanen et al. 1981), and facilitation (Gough et al. 2016). While there is a rich body of theoretical and empirical literature exploring of the effects of resource supply on ecological dynamics, most of this work has focused on free-living organisms (Tilman 1982, Holt et al. 1994, Miller et al. 2005). However, pathogens, parasites, and other symbionts comprise a large proportion of the diversity on earth (Lafferty et al. 2006, Qin et al.

48 2010), and the resources consumed by hosts, i.e., host nutrition, is an important mediator of
49 resource supply to these organisms (Smith et al. 2005). Over 20 year ago, Smith and Holt (1996)
50 used resource-competition theory to explore the effects of resource supply rate on pathogen-
51 pathogen interactions, but there have been few empirical extensions since the publication of this
52 seminal work (but see Chant and Gbaja 1985, Lacroix et al. 2014, 2017, Wale et al. 2017a,
53 2017b). In addition, theory in disease ecology has focused on among-host transmission and host
54 population dynamics and typically assumes that within-host processes reach an instantaneous
55 equilibrium (Keeling and Rohani 2008). Recently, Strauss et al. (2019) developed a
56 metacommunity model that combines the within-host pathogen dynamics explored by Smith and
57 Holt (1996) with an iconic two-pathogen, disease-ecology model (May and Nowak 1994) and
58 demonstrated that within-host pathogen-pathogen interactions can have strong effects on
59 pathogen dynamics at the host population scale. Here, we experimentally tested the effects of
60 host nutrition on the within-host population dynamics and among-host transmission rates of two
61 plant viruses in a grass host.
62 Pathogens can interact with one another through a variety of mechanisms within plants
63 (Sofonea et al. 2017), many of which can depend on resource availability. They can compete for
64 resources, such as host cells or materials for replication (e.g. proteins and ribosomes, Smith and
65 Holt 1996). For example, drug-resistant and susceptible malaria strains compete for pABA
66 within mice, which can be manipulated through diet (Wale et al. 2017a, 2017b). Pathogens also
67 can interact through the host's immune system, triggering or suppressing immune reactions
68 (Ezenwa et al. 2010). Immune functioning in mammals depends on vitamins, zinc, iron, and
69 iodine (Katona and Katona-Apte 2008), and plant susceptibility to certain pathogens can depend
70 on the C:N of nutrients in the soil (Hoffland et al. 1999). Pathogens also can produce molecules

71 that are toxic (i.e., spite) or can be used by other pathogens (i.e., common goods) (Abdullah et al.
72 2017). The production of such molecules depends on the availability of resources such as
73 nitrogen and carbon (Brown et al. 2009). Therefore, host diet may modify pathogen-pathogen
74 interactions through resource competition, immune-mediated interactions, and the exchange of
75 molecules.

76 Within-host, pathogen-pathogen interactions can affect overall pathogen fitness by
77 altering host population-scale rates such as transmission, recovery, and mortality (Mideo et al.
78 2008). Pathogens that reach higher densities within hosts are more likely to produce more
79 propagules for direct or vector-assisted transmission (Handel and Rohani 2015). In addition,
80 infections that last longer provide more opportunities for transmission to occur. In many cases,
81 within-host pathogen densities predict the probability of transmission given host-to-host contact,
82 but this relationship is not universal (Froissart et al. 2010, Handel and Rohani 2015). For
83 example, vector feeding time (Power et al. 1991) and pathogen-vector compatibility (D'Arcy and
84 Burnett 1995) also can affect the rate of transmission. Nutrients can mediate vector preference
85 for hosts and the amount of time a vector spends feeding on hosts (Nowak and Komor 2010),
86 which may alter how sensitive pathogen transmission is to within-host interactions. Pathogens
87 can also interact with one another within vectors, interfering with pathogen-vector compatibility
88 (Wen et al. 1991). The nutrition of hosts or vectors may affect this process.

89 A well-studied group of viruses that infects crops and wild plants, the Barley and Cereal
90 Yellow Dwarf Viruses (B/CYDVs, D'Arcy and Burnett 1995, Power et al. 2011), provide an
91 ideal system for investigating how nutrient availability mediates the outcome of pathogen-
92 pathogen interactions through within-host virus densities and transmission between hosts.
93 Nitrogen (N) can promote B/CYDV within-host density through increased host size (Whitaker et

94 al. 2015, but see Lacroix et al. 2017) and alleviate antagonistic interactions during co-inoculation
95 of two B/CYDVs (Lacroix et al. 2014). In addition, the composition of virus particles and the
96 processes of replication and transmission likely require similar resources across B/CYDVs
97 (D'Arcy and Burnett 1995, Sterner and Elser 2002). Therefore, it is likely that B/CYDVs
98 compete for resources within a shared host. Further, N and P can affect B/CYDV infection
99 prevalence in grasslands (Seabloom et al. 2010, 2013, Borer et al. 2014), which may be driven
100 by nutrient effects on within-host virus density, vector preferences for hosts, or vector population
101 growth rates (Mordecai et al. 2016, Shaw et al. 2017).

102 Here, we tested the hypothesis that host nutrition can constrain virus-virus interactions by
103 simultaneously determining both within-host density and transmission to new hosts. In a growth
104 chamber experiment, we manipulated soil N and P concentrations and quantified the resulting
105 interactions between two B/CYDVs within plants and during transmission between plants.
106 Consistent with models of pathogen-pathogen resource competition, we expected that nutrient
107 limitation would decrease the within-host density of the inferior competitor in coinfected plants
108 relative to singly infected plants and could ultimately lead to competitive exclusion (Tilman
109 1982, Smith and Holt 1996, Strauss et al. 2019). We expected that within-host competition
110 would affect transmission rates through links between within-host dynamics and transmission
111 (Handel and Rohani 2015, Strauss et al. 2019), but that transmission also would depend on
112 pathogen-density-independent vector processes (Power et al. 1991, D'Arcy and Burnett 1995).
113

114 **Methods**

115 The B/CYDV group is capable of infecting many grass species in locations around the
116 world (D'Arcy and Burnett 1995), making it likely that these viruses encounter a range of plant

117 N and P concentrations. The single-stranded RNA viruses are persistently transmitted by grass-
118 feeding aphids and the two virus species we used in this study, BYDV-PAV (PAV, hereafter)
119 and CYDV-RPV (RPV, hereafter) can both be vectored by the aphid species *Rhopalosiphum*
120 *padi* (D'Arcy and Burnett 1995). We maintained cultures of PAV and RPV in *Avena sativa* L.
121 cv. Coast Black Oat (National plant germplasm system, USDA) by transferring *R. padi* aphids
122 between infected and healthy *A. sativa* every three weeks (Methods S1). *R. padi* colonies were
123 maintained on live *A. sativa* plants with mesh cages. We obtained the virus isolates from Dr.
124 Stewart Gray at Cornell University (Ithaca, NY, USA), and the aphids from Dr. George Heimpel
125 at the University of Minnesota (St. Paul, MN, USA), who each collected these organisms in their
126 respective states. Healthy plants, infected plants, and plants with *R. padi* were grown in Sunshine
127 MVP potting soil (Sun Gro Horticulture, Agawam, MA, USA) and kept in separate growth
128 chambers at 20ºC with a 16:8 h light:dark cycle for one year prior to the experiment.
129 The experiment was carried out from February to August 2014 over five temporal blocks
130 (due to space and labor constraints). Variation among temporal blocks was examined in
131 statistical analyses and included in models when appropriate. *A. sativa* seeds were germinated in
132 164 mL conical pots with 70% Sunshine medium vermiculite (vermiculite and <1% crystalline
133 silica; Sun Gro Horticulture) and 30% Turface MVP (calcined clay containing up to 30%
134 crystalline silica; Turface Athletics, Buffalo Grove, IL, USA) that had been saturated with tap
135 water. Beginning two days after planting, we watered each plant with one of four modified
136 Hoagland solutions (7.5 μM N and 1 μM P ("Low"), 7.5 μM N and 50 μM P ("P"), 375 μM N
137 and 1 μM P ("N"), or 375 μM N and 50 μM P ("N+P"), Table S1), which have been shown to
138 affect plant growth and virus inoculation success (Hoagland and Arnon 1938, Seabloom et al.
139 2011, Lacroix et al. 2014). Plants were watered with 30 mL of nutrient solution twice per week

140 prior to inoculation and weekly following inoculation. After inoculation, plants were moved to a
141 growth chamber maintained at 20ºC with a 16:8 h light:dark cycle under 28W bulbs. Sample
142 sizes are reported in Table S2.

143 We inoculated the first set of plants ("source plants") ten to eleven days post planting
144 with PAV, RPV, or both using ten aphids per plant that fed on our virus cultures (PAV: five
145 aphids carrying PAV, five mock-inoculated; RPV: five carrying RPV, five mock-inoculated;
146 both: five carrying PAV, five carrying RPV, Methods S1). We destructively harvested the source
147 plants at eight different days post inoculation (DPI): 5, 8, 12, 16, 19, 22, 26, or 29 days. We
148 weighed the stems and leaves and stored approximately 60% of the tissue at -80ºC and used the
149 rest to measure transmission to four "receiving plants" grown in each of the four nutrient
150 treatments (Methods S1). The receiving plants were harvested 14-15 DPI and all of the stem and
151 leaf tissue was stored at -80ºC. On the first harvesting day, source plants weighed 0.16 g ± 0.006
152 g (1 s.e., n = 79, fresh wet weight) and had, on average, two leaves. By the final harvesting day,
153 they weighed 0.34 g ± 0.006 g (1 s.e., n = 92, fresh wet weight) and had, on average, three
154 leaves.

155 To quantify virus densities (number of viruses per mg plant) in source plants, we first
156 extracted the total RNA from approximately 50 mg of thawed plant tissue from the portion
157 stored at -80ºC. We then used one-step reverse transcription-quantitative polymerase chain
158 reaction to obtain estimates of the number of copies of PAV and RPV RNA within each sample
159 (RT-qPCR, see Methods S2 and Table S3 for more details). Virus densities greater than zero and
160 quantified by RT-qPCR methods were considered indicators of successful infection. We used
161 RT-PCR and gel electrophoresis to test the transmission success of receiving plants (see Lacroix
162 et al. 2014 for further details). Plants with accidental infections (i.e. PAV detected in RPV-

163 inoculated plants and RPV detected in PAV-inoculated plants, Table S2) were included in
164 analyses as singly infected plants because the timing of the accidental infection was unknown.
165 Such infections may have been caused by small aphids that escaped inoculation cages (Methods
166 S1).

167 We performed all statistical analyses in R version 3.5.2 (R Core Team 2018). To analyze
168 the effects of nutrient addition and coinfection on virus density in source plants, the infection rate
169 of source plants, and transmission to receiving plants, we fit Bayesian generalized linear models
170 to data for each virus species using the brms package (Bürkner 2017). Virus density was
171 modeled with a Gaussian distribution and formatted as ln(density) ~ coinfection * N addition * P
172 addition, where coinfection, N addition, and P addition were binary variables (0 or 1, applies to
173 all other models). Harvesting day was included as a first-order autocorrelation structure (cor_ar
174 function). Source plant infection was modeled with a Bernoulli distribution (logit-link) and
175 formatted as infection ~ coinfection * N addition * P addition. Harvesting day was included as a
176 random effect because autocorrelation structures were not compatible with this family of models.
177 Transmission was also modeled with a Bernoulli distribution (logit-link) and formatted as
178 transmission ~ density + coinfection * (N_{source} * P_{source} + $N_{receiving}$ * $P_{receiving}$), where the subscripts
179 indicate which plant the nutrient treatment was applied to, and density was centered and scaled.
180 Harvesting day and experimental block were included as crossed random effects. We used data
181 from a previous experiment that measured PAV and RPV densities and transmission under
182 almost identical laboratory conditions to inform the priors for the density and transmission
183 models (Table S4, Lacroix et al. 2017). All models were run with three Markov chains, 6000
184 iterations each with a 1000 iteration warm-up. We used r-hat values, visualizations of chain
185 convergence, and visual comparisons of the observed data and simulated data from the posterior

predictive distributions to evaluate model fit. Models with informative and uninformative priors had similar fits and produced similar estimates (Fig. S1). In the results, we present the estimated means and 95% highest posterior density intervals (HDI) from models with informative priors.

Results

The proportion of plants successfully infected with PAV increased over the first three harvesting days (Fig. S2A) while RPV infection was relatively high over time (Fig. S2B). In successfully infected plants, virus density varied over time, with a general increase over the first three harvesting days, especially for RPV (Fig. 1A, B). The following results are consistent whether or not the first two harvesting days are included in the analysis (Fig. S3). Here, we present results from the full dataset (Table S4).

Coinfection had the most dramatic effect on PAV when plants were grown with low nutrients, increasing PAV density by 100% (95% HDI: -8.4%, 221%; Fig. 1C). However, coinfection decreased PAV density when plants were grown with both nutrients (-31.0%, 95% HDI: -69.0%, 150%). PAV and RPV densities in the same plant were positively correlated across all nutrient treatments ($\rho = 0.25$, 95% CI: 0.0015, 0.47, df = 60, Fig. 2). In contrast, co-inoculation had a consistent negative effect on PAV infection, with the greatest reduction when plants were grown with low nutrients (Fig. S2C).

Coinfection had a slightly positive effect on RPV density when plants were grown with low nutrients (increased 32.5%, 95% HDI: -45.2%, 123%), but the largest effect was with N addition (density increased 112%, 95% HDI: -31.6%, 280%; Fig. 1D). P addition led to a slightly negative impact of coinfection on RPV density (-24.8%, 95% HDI: -73.0%, 39.4%). Simultaneous N and P addition increased RPV density in both single and coinfection. However,

209 this effect was driven by P in single infection and N in coinfection. The effects of co-inoculation
210 on RPV infection mirrored the impacts of coinfection on RPV density (Fig. S2D).

211 The probability of transmission tended to increase over the first three harvesting days for
212 both viruses (Fig. 3A, B). A notable exception, however, is RPV transmission from singly
213 infected plants, which took up to five harvesting days to increase (Fig. 3B). Despite the similarity
214 in these trends to patterns of virus density (Fig. 1A, B), the relationship between within-host
215 density and transmission was weak for PAV (Fig. 4A). RPV transmission, however, increased
216 with within-host density (Fig. 4B).

217 Nutrient addition to the source plant, especially N, tended to increase PAV transmission
218 from singly-infected plants (Fig. 3B). However, coinfection decreased PAV transmission by up
219 to 34.5% when the source plant received N addition (95% HDI: -58.0%, -11.7%). Coinfection
220 had a slightly positive effect on PAV transmission when the receiving plant was grown with
221 elevated P, increasing the transmission rate by up to 30.0% (95% HDI: 3.7%, 56.6%), but this
222 was primarily driven by decreased single infection transmission rather than increased coinfection
223 transmission.

224 Coinfection had a large positive effect on RPV transmission, increasing it by up to 69.6%
225 (95% HDI: 54.1%, 83.4%; source plant = N + P, receiving plant = P, Fig. 3D). In general, P
226 addition to the source plant increased transmission from coinfected, but not singly infected plants
227 (Fig. 3D). Similarly, nutrient addition to the receiving plants increased RPV transmission from
228 coinfected plants but decreased it from singly infected plants (Fig. 3D).

229

230 **Discussion**

231 Coinfection by multiple pathogens is more the rule than the exception, and pathogen
232 interactions can alter disease prevalence and severity (Seabloom et al. 2015, Tollenaere et al.
233 2016, Hassani et al. 2018). In addition, host nutrition can mediate many host-pathogen
234 interactions (Smith et al. 2005) and may determine the frequency and impacts of coinfection
235 (Borer et al. 2016, Sofonea et al. 2017, Strauss et al. 2019). Here, we examined the impacts of N
236 and P availability on virus-virus interactions during two phases: within-host reproduction and
237 among-host transmission. Within the grass host, viruses interacted through both antagonistic and
238 facilitative processes, which were dependent on nutrient availability. Virus interactions during
239 transmission also depended on host nutrients but not within-host interactions (Fig. 5). Together,
240 these results suggest that nutrient availability influences pathogen-pathogen interactions through
241 multiple simultaneous processes.
242 We hypothesized that PAV and RPV would compete for N and P based on multiple lines
243 of evidence: B/CYDVs likely require similar resources for replication and transmission (D'Arcy
244 and Burnett 1995, Sterner and Elser 2002), N and P additions have promoted PAV and RPV
245 infection prevalence and within-host PAV density (Seabloom et al. 2010, 2013, Borer et al.
246 2014, Whitaker et al. 2015), and N addition has eased negative interactions between PAV and
247 RPV (Lacroix et al. 2014). Resource competition can lead to more positive interactions with
248 elevated resources due to lower intensity of competition, or more negative interactions with
249 elevated resources due to loss of niche dimensionality (Tilman 1982). We found evidence for
250 resource competition between PAV and RPV. In all cases, RPV had a negative effect on PAV
251 (infection, density, and transmission; Fig. 5). Adding nutrients tended to decrease PAV density
252 in and transmission from coinfected hosts, suggesting that PAV may be a superior competitor for
253 N and P and that adding these resources removed its competitive advantage (Tilman 1982,

254 Harpole et al. 2016). A major constraint to attributing resource competition to these effects is
255 that N and P did not seem to be strongly limiting resources for the viruses in our experiment;
256 elevated nutrients did not enhance virus densities in singly-inoculated plants except in the case of
257 P addition for RPV. In contrast to our hypothesis, resource competition was not the dominant
258 force structuring virus-virus interactions because the observed antagonistic interactions may be
259 explained by other processes and resource-mediated facilitation was pervasive (Fig. 5).
260 Mechanisms other than resource competition may be appropriate for describing nutrient-
261 mediated interactions among B/CYDVs. Plant immunity against viruses consists of multiple
262 pathways that are triggered by different viral characteristics (Mandadi and Scholthof 2013).
263 Immune responses that target both viruses may explain negative interactions between PAV and
264 RPV (Abdullah et al. 2017). B/CYDVs possess mechanisms to suppress RNA silencing, a plant
265 immune mechanism. Complementarity between silencing suppressors may explain facilitation
266 between PAV and RPV (Liu et al. 2012, Almasi et al. 2015). Another source of facilitation may
267 be the production of public goods (Wen and Lister 1991). Such goods may be important for
268 replication, transmission, or both, and may explain incongruencies between nutrient effects on
269 interactions within the host and during transmission. While some B/CYDVs interact within aphid
270 vectors, competing for receptor sites, this has not been previously observed for PAV and RPV
271 (Wen et al. 1991). However, inconsistencies between the results from this experiment and
272 previous studies on B/CYDVs may be due to the nutrient-dependency of virus-virus interactions.
273 Exploring the mechanistic underpinnings of nutrient-dependent PAV and RPV interactions
274 would help clarify the causes of these discrepancies and inform a more general predictive
275 framework for resource-mediated pathogen-pathogen interactions.

276 Strauss et al. (2019) demonstrated that the rate of competitive exclusion of pathogens
277 within hosts relative to host lifespan can affect pathogen densities and disease prevalence. We
278 did not observe competitive exclusion of either virus over 29 days post inoculation and the
279 population growth rates of both viruses appeared to equilibrate. In addition, the prevalences of
280 PAV and RPV in a perennial grass increased over a four-year grassland study (Seabloom et al.
281 2009), suggesting long-term co-occurrence of these two viruses. Extending the length of the
282 present experiment or performing mutual invasibility trials would help confirm the stability of
283 the two-pathogen system (Chesson 2008). Due to fast within-host dynamics relative to host
284 lifespan and no competitive exclusion, PAV and RPV would be expected to reach higher within-
285 host densities, but lower prevalences, than "slow-dynamic" competitive pathogens (Strauss et al.
286 2019), particularly in grass species with infection-induced mortality (Malmstrom et al. 2005).
287 However, multiple aspects of PAV and RPV interactions remain unknown. Because a large
288 proportion of PAV infections were so low in concentration that they could not be reliably
289 quantified, it is unclear whether early dynamics between PAV and RPV include failed
290 colonization or competitive exclusion. Further, quantifying virus density in vectors and plants
291 that have received transmission could help identify the stage of transmission at which nutrients
292 are most influential.

293 Nutrient-mediated, virus-virus interactions had differential effects on two closely related
294 viruses, and these effects changed during the two phases of infection that we examined (within-
295 host interactions and transmission). The metacommunity framework is a useful starting point to
296 understand disease dynamics, where hosts serve as patches, within-host density is analogous to
297 within-patch population density, and transmission is analogous to dispersal (May and Nowak
298 1994, Borer et al. 2016). However, our experiments demonstrate that transmission does not

always depend on within-host density, a common assumption in metacommunity models (Leibold et al. 2004). In addition, it is clear from our results that nutrients can mediate pathogen-pathogen interactions through more mechanisms than only resource competition. Building on existing theories of pathogen-pathogen resource competition (e.g., Smith and Holt 1996) to include facilitation and immune-mediated interactions would improve predictions about multi-pathogen infections. For example, models that predict interactions among cross-feeding microbes (e.g., Harcombe et al. 2014) could be integrated into disease ecology models and models that include resource-dependent immunity (e.g., Cressler et al. 2014) could be expanded to include pathogen-pathogen interactions.

Motivated by community ecology theory, we found that nutrient supply to hosts (i.e., host diet) can impact pathogen interactions. In addition to finding evidence for nutrient-dependent antagonistic interactions within a grass host, the two-virus system demonstrated nutrient-dependent facilitation and interactions during transmission that were not predicted by within-host dynamics. These unexpected findings can help shape the application of community ecology theory to disease systems. This study also contributes to a growing literature on the impacts of resources on host-associated microbial communities (Griffin et al. 2017, Berg and Koskella 2018, Reese et al. 2018), which can inform a breadth of practices ranging from medicine to agriculture. Strengthening the linkages between community ecology and disease ecology will aid the advancement of both fields and their relevant applications.

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501

502 **Data availability**

503 Code is available in the first author's GitHub repository (https://github.com/aekendig/nutrients-
504 plant-viruses) and will be archived with data at https://portal.edirepository.org.

505 **Figure legends**

506 **Figure 1.** The effects of nutrient addition (N, P, or both) and coinfection on the log-transformed
507 density of (A) PAV and (B) RPV measured over time (days post inoculation). Points and error
508 bars represent mean +/- 1 SE. The model estimated mean and 95% HDI of log-transformed (C)
509 PAV and (D) RPV density for each inoculation and nutrient treatment, pooled over time.

510

511 **Figure 2.** Log-transformed PAV and RPV density in the same coinfected plants.

512

513 **Figure 3.** The effects of nutrient addition to the source plant (N, P, or both) and coinfection on
514 the probability of (A) PAV and (B) RPV transmission measured over time (days post
515 inoculation). Points and error bars represent mean +/- 1 95% CI. The model estimated mean and
516 95% HDI of (C) PAV and (D) RPV transmission for each inoculation and receiving plant
517 nutrient treatment, pooled over time. Points are colored by the source plant nutrient treatment.

518

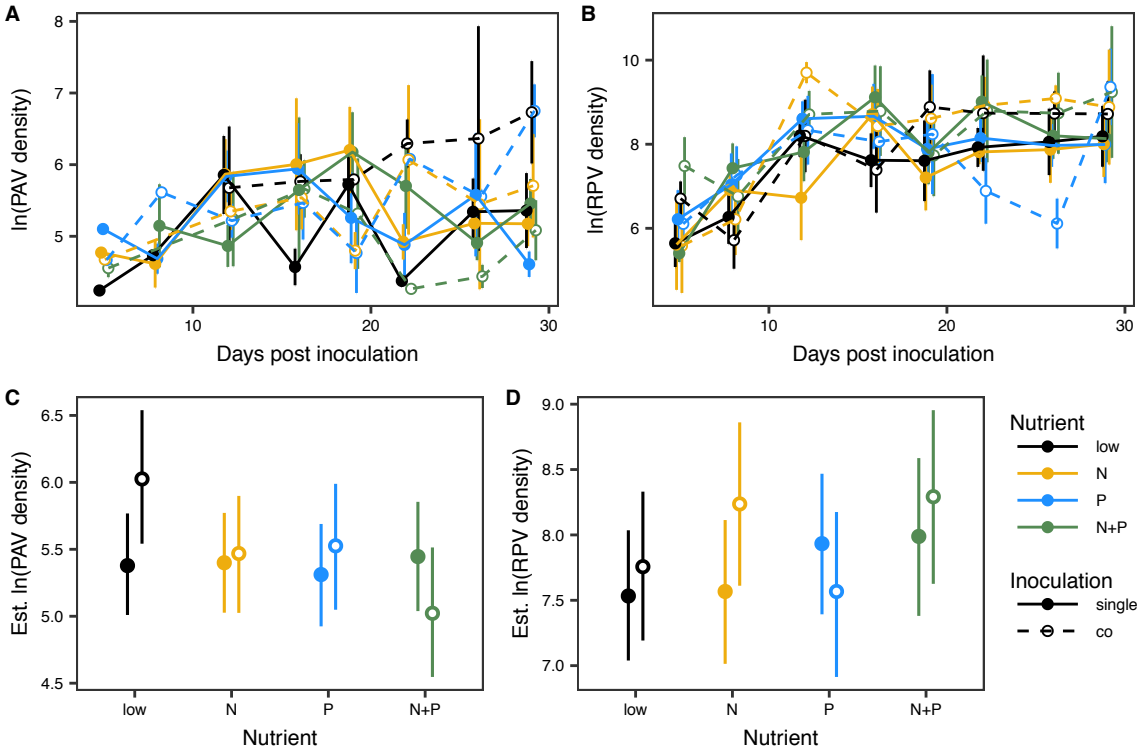
519 **Figure 4.** The relationship between virus density and the probability of transmission for (A)
520 PAV and (B) RPV after accounting for impacts of coinfection and nutrient addition (Fig. 3).
521 Points represent raw data, colored by the nutrient treatment of the source plant. Lines and shaded
522 intervals represent the model estimated mean and 95% HDI, respectively.

523

524 **Figure 5.** Graphical summary of results, including the within-host densities and transmission
525 rates of PAV (left side of plants) and RPV (right side of plants). The single infection baseline
526 depicts the density of each virus within singly-infected plants (filled area relative to leaf area)
527 and the transmission rate between plants (thickness of arrows). It does not correspond to any

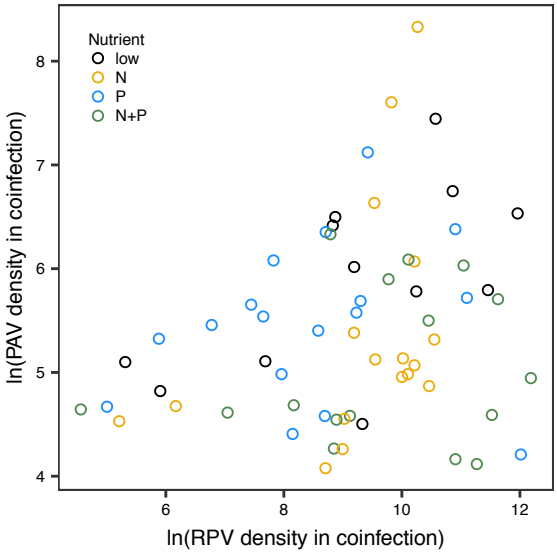
528 particular nutrient treatment but shifts in the values it represents for each nutrient treatment. Low
529 nutrients, N addition, P addition, and N + P addition depict the changes in virus density and
530 transmission due to coinfection under each nutrient treatment relative to the single infection
531 baseline. The source and receiving plants have the same nutrient treatment. Larger areas
532 (accompanied by +) and larger arrows (accompanied by + or ++) indicate that coinfection
533 benefitted the virus through density or transmission, respectively. Smaller areas (accompanied by
534 -) and smaller arrows (accompanied by -) indicate that coinfection suppressed the virus. No
535 effect of coinfection is represented by areas and arrows the same size as the single infection
536 baseline (accompanied by 0).

537 **Figure 1**



538

539 **Figure 2**



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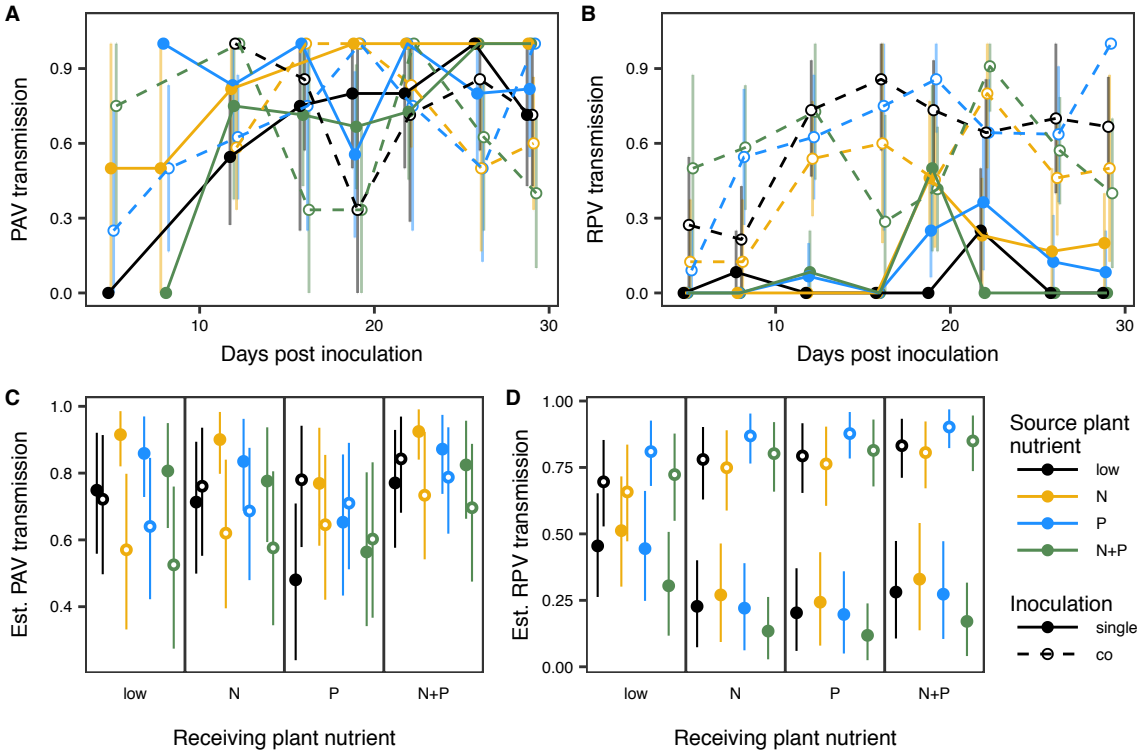
541 **Figure 3**



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543 **Figure 4**

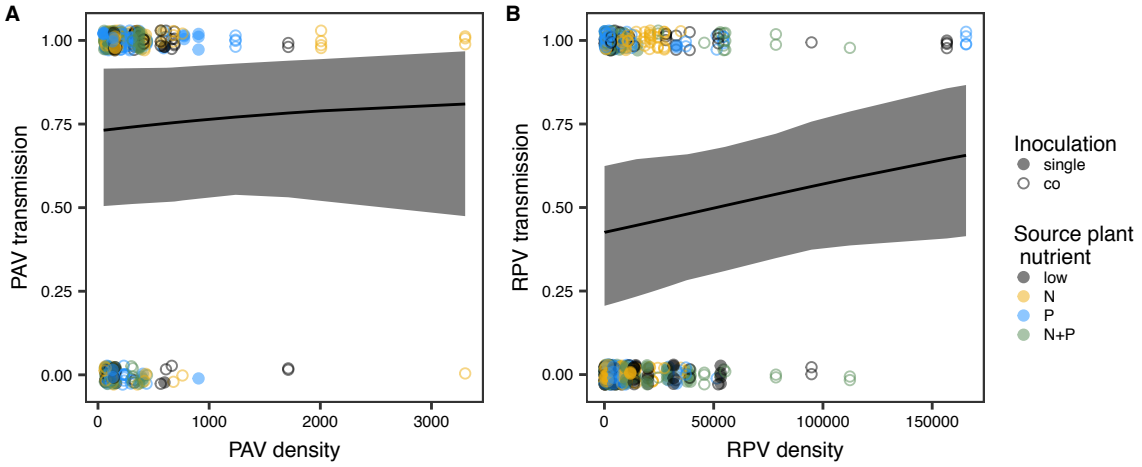

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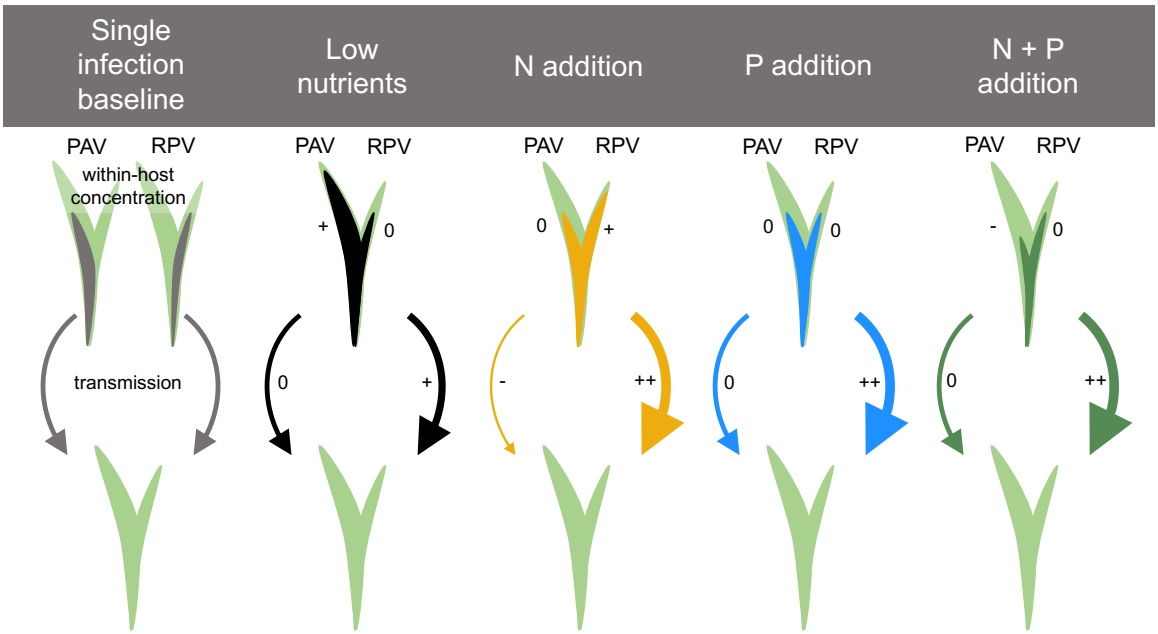
Figure 5


Single infection baseline
Low nutrients
N addition
P addition
N + P addition
PAV
RPV
within-host concentration
transmission
+
0
-
++