

Direct and insect-mediated effects of pathogens on plant growth and fitness

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Abstract

1. Plants are attacked by a large diversity of pathogens. These pathogens can affect plant growth and fitness directly but also indirectly by inducing changes in the host plant that affect interactions with beneficial and antagonistic insects. Yet, we lack insights into the relative importance of direct and indirect effects of pathogens on their host plants, and how these effects differ among pathogen species.
2. In this study, we examined four fungal pathogens on the wood anemone *Anemone nemorosa*. We used field observations to record the impacts of each pathogen species on plant growth and fitness throughout the season, and experimental hand pollination and insect feeding trials to assess whether fitness impacts were mediated by pathogen-induced changes in plant–pollinator and plant–herbivore interactions.
3. Three out of four pathogens negatively affected plant size, and pathogens differed strongly in their effect on plant architecture. Infected plants had lower fitness, but this effect was not mediated by pollinators or herbivores. Even so, two out of four pathogens reduced herbivory on anemones in the field, and we found negative effects of pathogen infection on herbivore preference and performance in feeding trials.
4. **Synthesis.** Our results are of broader significance in two main respects. First, we demonstrated that pathogens negatively affected plant growth and fitness, and that the magnitude of these effects varied among pathogen species, suggesting that pathogens constitute important selective agents that differ in strength. Second, direct effects on plant fitness were more important than effects mediated by beneficial and antagonistic insects. In addition, although we did not detect insect-mediated effects on plant fitness, the negative effects of some pathogens on herbivore preference and performance indicate that pathogen communities influence the distribution and abundance of herbivores.

KEYWORDS

Anemone nemorosa, insect herbivores, plant performance, plant–pathogen–insect interactions, pollinators, rhizomatous clonal plants, systemic fungal pathogens

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1 | INTRODUCTION

Plants are attacked by a large diversity of pathogens (Lucas, 2020). These pathogens can impact plant growth and fitness directly (Goss & Bergelson, 2007; Meléndez & Ackerman, 1993), but also indirectly by inducing changes that alter plant–insect interactions (Shikano et al., 2017; Stevenson et al., 2017). Yet, studies to date have largely ignored the effects mediated by beneficial or antagonistic insects (Burdon, 1991; Jarosz & Davelos, 1995; Mordecai, 2011), and we still know little about the extent to which total effects of pathogens on plant performance are direct versus indirect. Moreover, the direction and strength of both direct and indirect effects on the host are likely to vary among pathogen species (Jarosz & Davelos, 1995; Mordecai, 2011). Despite this, the majority of studies have focussed on the direct impact of a single pathogen species on plant growth or fitness, and our ability to assess the range of direct and indirect effects exerted by different pathogen species on a given host plant is thus limited. To better understand the impact of pathogens on the ecology and evolution of plant populations, it is essential to assess the direct and indirect effects of the diversity of pathogens associated with a given host plant species (Dobson & Crawley, 1994).

Pathogens can directly affect plant growth and fitness by modifying resource availability, metabolism and physiology of their host plant (Burdon, 1987). Infected plants are expected to have reduced resources for growth and reproduction as defence is costly and pathogens withdraw nutrients from the host (Berger et al., 2007; Denancé et al., 2013). However, due to a diversity of molecular and physiological plant responses upon pathogen infection (Berger et al., 2007), the strength and direction of effects of pathogens on plant growth and fitness vary greatly (Jarosz & Davelos, 1995; Kluth et al., 2005). The elicitors that pathogens release upon invasion can interfere with the primary metabolism of the host plant and photosynthetic activity can be reduced (García-Brugger et al., 2006), which might limit plant growth (Scharte et al., 2005; Swarbrick et al., 2006). Plants induce different defence-related hormonal pathways in response to different pathogen species (Glazebrook, 2005), and pathogens frequently manipulate plant hormonal pathways to promote the spread of infection (López et al., 2008). For example, some pathogens stimulate auxin signalling to induce plant growth while suppressing plant defences (Chen et al., 2007; López et al., 2008). Other pathogens manipulate plant hormones to abort or inhibit flower production, thereby severely hampering plant fitness (Dermastia, 2019; Minato et al., 2014). Plant fitness can also be reduced by pathogen-induced effects on investment in seed production (Denancé et al., 2013; Heil et al., 2000). Insights into the different ways through which pathogens directly affect plant growth and fitness are vital to understand their impact on the dynamics and evolutionary trajectories of plant populations.

The plant responses triggered by pathogen infection can also interfere with mutualistic interactions, such as plant pollination, or antagonistic interactions, such as plant herbivory (Chakraborty & Roy, 2021; Noman et al., 2020). Nevertheless, only a few studies to date investigated insect-mediated effects of microbes on plant

fitness, of which four studies focussed on soil and arbuscular mycorrhizal fungi, one on a viral pathogen, and none on fungal pathogens (Table S1). Pathogens can change the attractiveness of a plant to pollinators by affecting traits such as flower height (Korves & Bergelson, 2003), flower morphology (Himeno et al., 2011), nectar production (Stevenson et al., 2017) or volatiles (Groen et al., 2016). Pathogens may also influence plant–herbivore interactions by changing plant traits that act as cues for insect herbivores (Grunseich et al., 2020), such as colour (Ajayi & Dewar, 1983), volatiles (Piesik et al., 2011; Ponzio et al., 2013), or size and growth pattern (García-Guzmán & Wennström, 2001; Wennström & Ericson, 1991). For example, ovipositing moths (beet armyworm *Spodoptera exigua*) were repelled by the olfactory cues of powdery mildew-infected roses *Podosphaera pannosa* (Yang et al., 2013). Such aversion for infected plants may be linked to impaired herbivore performance, for example, due to lower nutrient contents or higher levels of defensive compounds in infected plant tissues (Dangl & Jones, 2001; Fernandez-Conradi et al., 2018; Yang et al., 2013). To understand the different ways in which pathogens can influence plant fitness, it is necessary to consider the interactions between plants and their beneficial and antagonistic insects. Moreover, pathogens that are more similar in their life-history traits (e.g. systemic pathogens) may induce similar plant responses, which may predictably affect plant–insect interactions. To link pathogen life histories to plant responses and insect-mediated effects on plant fitness, we therefore need to explore plant responses to multiple pathogen species and investigate how these responses influence interactions with mutualistic and antagonistic insects.

The overarching aim in this study was to examine the impact of multiple pathogens on the growth and fitness of a common host plant, and assess to what extent these effects are direct versus mediated by changes in interactions between plants and mutualistic and antagonistic insects (Figure 1). To this aim, we studied four fungal diseases commonly found on the wood anemone *Anemone nemorosa*, a perennial forest herb, and addressed the following questions:

1. What are the effects of fungal pathogens on plant growth and fitness?
2. To what extent are effects on plant fitness mediated by interactions with pollinators and herbivores?
3. Does the relative importance of direct versus indirect effects differ among pathogen species?

We used field observations to relate pathogen infection to plant growth and fitness, a hand-pollination experiment to identify pollinator-mediated effects, and a combination of field observations and feeding trials to identify herbivore-mediated effects. We hypothesized that fungal pathogens have direct negative effects on plant growth and fitness (Figure 1). We hypothesized that infected plants are less attractive to pollinators than healthy plants, leading to an indirect negative effect of infection on plant fitness. We also hypothesized that herbivores have a lower preference for infected

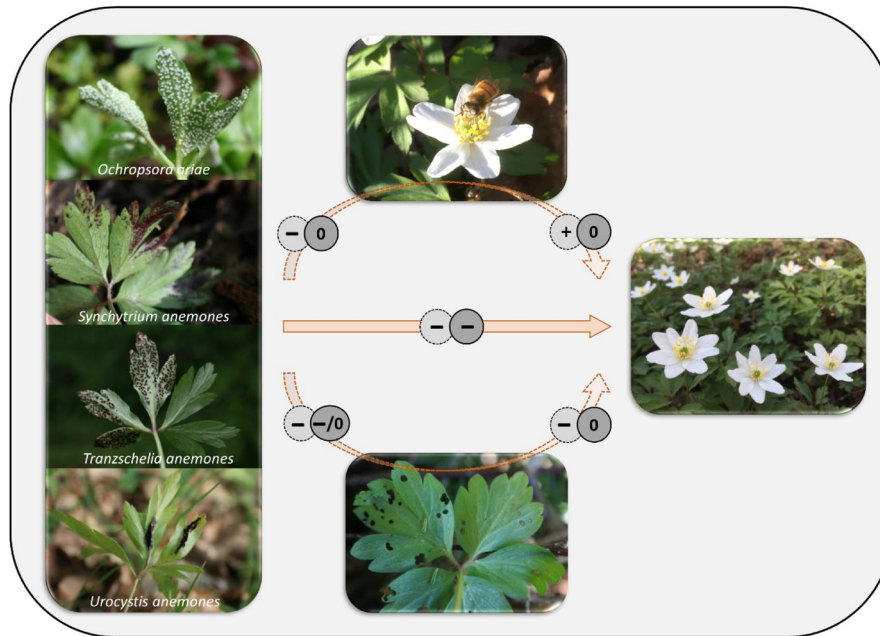


FIGURE 1 The potential direct (solid arrow) and indirect (dashed arrows) effects of plant pathogens (left: *Ochropsora ariae*, *Synchytrium anemones*, *Tranzschelia anemones* and *Urocystis anemones*) on the growth and fitness of *Anemone nemorosa*. Predicted effects are shown in dashed, light grey circles, and observed effects in solid, dark grey circles, with ‘-’ denoting a negative effect, ‘+’ a positive effect and ‘0’ no effect. As expected, pathogens had a direct, negative effect on plant growth and fitness, though the magnitude of the negative effect differed among pathogens. In contrast to our expectation, there was no mediating effect of pollination on plant fitness. As predicted, some of the pathogens negatively affected herbivore preference and performance and reduced herbivory, but we found no herbivore-mediated effect of pathogens on plant fitness

plants as well as a reduced performance on these plants. Due to this lower preference and assumed negative effects of herbivory on plant fitness, we predicted pathogens to have a positive indirect effect on plant fitness by reducing herbivory. Based on differences in the effects of pathogens on plant metabolism and hormone signalling, we predicted that the magnitude and relative importance of direct and indirect effects on plant growth and fitness vary among pathogen species. Pathogens that affect plant traits that are potentially important as cues to herbivores and pollinators (e.g. plant size or flower height) were expected to induce the strongest insect-mediated impacts on plant fitness.

2 | MATERIALS AND METHODS

2.1 | Study system

The wood anemone *Anemone nemorosa* is a perennial herb that is common throughout European forests, although populations get smaller and sparser at the range edge in northern Scandinavia (Shirreffs, 1985). Wood anemones reproduce clonally through the growth of the rhizome and sexually through the production of seeds (Ernst, 1983). In early spring, wood anemones develop a shoot of around 5–15 cm tall with lobed leaves from a bud on the rhizome. Several herbivorous insects, such as larvae of the sawfly *Monophadnus pallescens*, attack the vegetative parts of the wood anemone. Around the beginning of May, anemones form

hermaphroditic flowers with white petals that are pollinated by flies, thrips, small beetles and bees, which use visual and/or floral scent cues to find flowers (Shirreffs, 1981, 1985). Pollinators are mainly rewarded with pollen, and only occasionally with nectar drops (Erbar & Leins, 2013). In Sweden, fertilized ovules turn into mature seeds around the beginning of June.

The four major fungal pathogens on the wood anemone are *Ochropsora ariae* (OA; *Pucciniomycetes*), *Synchytrium anemones* (SA; *Chytridiomycetes*), *Tranzschelia anemones* (TA; *Pucciniomycetes*) and *Urocystis anemones* (UA; *Ustilaginomycetes*; Figure 1; Hylander et al., 1953; Lindeberg, 1959). OA is a perennial systemic rust fungus and spreads in the rhizome of anemones. Anemones infected with OA typically have elongated shoots and rarely flower (García-Guzmán & Wennström, 2001; Wilson & Henderson, 1966). OA needs two host plants to complete its sexual cycle. In spring, the fungus produces white aeciospores on the abaxial side of anemone leaves (Figure 1), and these spores infect the rowan *Sorbus aucuparia* (Gjærum, 1974). On OA-infected rowan, teliospores mature during autumn, and basidiospores infect the growing tips of *A. nemorosa* rhizomes the next spring. SA is a non-systemic chytrid fungus that appears as black-violet lesions on leaves and stems (Figure 1). TA is a perennial systemic rust fungus (*Pucciniomycetes*), causing lesions with brown spores on the abaxial side of anemone leaves (Figure 1). Like OA, infection by this pathogen typically leads to elongated shoots without flowers (García-Guzmán & Wennström, 2001). UA is annual systemic smut fungus that forms blisters on the leaves and stems of *A. nemorosa*, which will burst open to release black-brown spores (Figure 1).

2.2 | Effects of pathogens on plant growth and fitness

To investigate whether pathogens affect plant growth and fitness, we established 'quadruples' of permanently marked anemone individuals consisting of two healthy plants and two plants infected by the same pathogen, growing within an area of maximum 1 by 1 m (Figure S1). The locations of all quadruples were picked randomly in a forest area of approximately 1 km² (around 59°21'49.3"N, 18°04'10.5"E, central Sweden, Stockholm). Once a location was chosen, we aimed to set up one quadruple for each disease at that location. To investigate the effect of disease on plant growth, we set up 25 quadruples for each of the four diseases in April 2019 (Figure S1a), resulting in a total of 100 quadruples (referred to as 'plant growth quadruples'). For each individual, we measured shoot height (stem length from the ground to the leaves), size of the largest leaf (length × width) and plant size ($\sqrt{\text{shoot height} \times \text{leaf size}}$) every week until anemones started to wither (end of May). In the rare event that an individual identified as healthy became infected during the experiment, we replaced this individual by a nearby healthy plant. To investigate the effect of disease on plant fitness, we set up 25 additional quadruples for both SA and UA diseases in April 2020 (Figure S1b), resulting in a total of 50 quadruples (referred to as 'plant fitness quadruples'). As TA-infected plants never produced a flower, and OA-infected plants only very rarely had a flower, we did not include plants with these two diseases in the fitness assessments. We quantified fitness as the total seed production for all individuals within these quadruples, as further described below.

2.3 | Pollinator-mediated effects of pathogens on plant fitness

We used the plant fitness quadruples to examine whether interactions with pollinators mediated effects of pathogens on plant fitness. One healthy plant and one infected plant in each quadruple were hand-pollinated, whereas the other healthy and infected plants experienced only ambient pollination (Figure S1b). We visited the quadruples weekly to observe the development stage of all marked individuals. When stigmas were receptive, we hand-pollinated by tipping the anthers of two pollen-producing anemone flowers to the stigmas of the recipient flower. To increase the likelihood of successful pollination, we repeated hand pollinations 7 days later. When seeds were fully developed, we counted the number of ovules and mature seeds, and measured shoot height, size of the largest leaf, plant size and height of the flowering stem (stem length from ground to the base of the flower).

2.4 | Herbivore-mediated effects of pathogens on plant fitness

To investigate whether pathogens influence the level of herbivory, we assessed foliar herbivory on plants within the plant growth

quadruples. During the entire growing season of anemone, we scored the percentage of leaf tissue that was removed by herbivores for each plant visually on a weekly basis, using five categories: (0) no damage, (1) <5% damage, (2) 5%–20% damage, (3) 20%–50% damage, and (4) >50% damage. To investigate whether interactions with herbivores mediated effects of pathogens on plant fitness, we assessed the amount of foliar herbivory on all plants also within the 'plant fitness quadruples' at the time of seed maturation, using the same scores.

To study whether the effect of pathogens on herbivory was due to changes in herbivore preference and performance, we conducted experiments with field-collected larvae of the sawfly *Monophadnus pallescens*. To examine the effect of pathogen infection on herbivore preference, we used a dual choice experiment where each larva ($n = 40$ larvae) was presented with one leaf disc taken from a healthy plant, and one leaf disc taken from an infected plant (OA, TA, SA or UA). Spores were visible on all leaf discs taken from infected plants. The 10-mm diameter leaf discs were placed 1 cm apart in the middle of a petri dish with moist filter paper, with the larva placed in between the two leaf discs (Figure S1c). Dual-choice trials lasted for 16 hr, during which we recorded the following: (a) First choice, the leaf disc from which the larva took the first bite; (b) 2-hr choice, the leaf disc from which the larva was feeding after 2 hr; (c) 2-hr consumption, the percentage of each of the two leaf discs consumed after 2 hr and (d) 16-hr consumption, the percentage of each of the two leaf discs consumed after 16 hr. Each larva was used in three trials in a random order to reduce potential biases due to prior feeding experience ($n = 30$ replicates per pathogen). To examine the effect of pathogen infection on herbivore performance, we used a no-choice experiment where each larva received a diet of either healthy leaves ($n = 10$ larvae), OA-infected leaves ($n = 13$ larvae), TA-infected leaves ($n = 11$ larvae) or UA-infected leaves ($n = 11$ larvae). We took care to keep levels of infection similar for all larvae within a treatment, and infection levels within treatments were representative of observed infection levels in the field. Due to a lack of larvae, we did not include a diet on SA-infected leaves. Larvae were kept individually in petri dishes with moist filter paper. Every other day, we provided the larvae with fresh leaves that were collected from anemones in the field. We measured larval weight every 2 days until the larva entered the prepupal stage.

2.5 | Statistical analyses

All statistical analyses were done in R v. 3.6.3 (R Core Team, 2020). We used the functions *lmer* and *glmer* in package *lme4* to fit (generalized) linear mixed-effect models (Bates et al., 2015) and the function *clmm* in package *ORDINAL* to fit models with an ordinal response variable (Christensen, 2019). Although some variables were used as both response and predictor, path models were not considered adequate since our analyses included ordinal variables (i.e. herbivory levels), combined datasets (2019 and 2020) and contained independent mediating factors (i.e. pollination treatments). We verified model assumptions using the packages *sjPlot*

and DHARMA (Hartig, 2020; Lüdecke, 2020), assessed significance of explanatory variables with the ANOVA function in the *CAR* package (Fox & Weisberg, 2019) and estimated pairwise comparisons with the *emmeans* function in the *EMMEANS* package (Lenth, 2020). For an overview of all examined questions, response variables and model structures, see Table S2.

2.5.1 | Effects of pathogens on plant growth and fitness

To examine the effect of pathogen infection on plant growth, we modelled shoot height, leaf size, plant size and flower height as functions of disease status (healthy or infected) and week, using pathogen-specific models. To account for temporal variation in the effect of pathogens on plant growth, we included the interaction between disease status and week. To account for repeated measures on the same plants, we included plant ID as a random intercept, and to account for the effect of location, we included quadruple ID as a random intercept. To identify the effect of pathogen infection on plant sexual reproduction and fitness, we modelled the number of ovules and number of mature seeds as functions of disease status (healthy or infected), using pathogen-specific models. We also included quadruple ID as a random intercept.

2.5.2 | Pollinator-mediated effects of pathogens on plant fitness

To examine whether interactions with pollinators mediated the effects of pathogens on plant fitness, we modelled seed set (i.e. the proportion of ovules that developed to mature seeds) as a function of disease status, pollination treatment (hand pollination vs. ambient pollination) and the interaction between disease status and pollination treatment in pathogen-specific models. A higher seed set for hand-pollinated plants than plants with ambient pollination would indicate that seed set is pollen limited, and an effect of disease status \times pollination treatment would indicate that the degree of pollen limitation differs between infected and healthy plants. To account for differences in plant condition, we included plant size. Plant size was scaled separately for each pathogen species to minimize confounding effects of disease on plant size and growth form. To examine whether pollinator preference was influenced by the height of the flowering stem, we included flower height, which was also scaled separately for plants attacked by different pathogen species. We included quadruple ID as a random intercept to account for location, and plant ID to account for overdispersion.

2.5.3 | Herbivore-mediated effects of pathogens on plant fitness

To investigate whether pathogen infection affected herbivory, we modelled the level of insect herbivory on anemones in the field

(ordinal variable with scores from 0 to 4) as a function of disease status and week. To account for temporal variation in the effect of pathogens on herbivory, we included the interaction between disease status and week. We included plant size (scaled separately for each pathogen species) in the model, as well as the interaction term plant size \times disease status, to examine whether the effect of plant size was dependent on disease status. To account for repeated measures of the same plants, we included plant ID as a random intercept, and to account for the effect of location, we included the random intercept quadruple ID. To test whether herbivory affected plant sexual reproduction and fitness, we modelled the number of ovules and mature seeds as functions of cumulative herbivory (amount of herbivory in the end of the season, scored in categories from 0 to 4), disease status and plant size (scaled separately for each pathogen species). To examine whether the impact of herbivory was dependent on disease status, we included the interaction disease status \times cumulative herbivory. The random intercept quadruple ID was included.

To examine whether changes in herbivory were due to preferences of herbivores towards healthy versus infected leaves, we modelled larval preference as a function of healthy versus infected leaf discs in pathogen-specific models. Response variables for larval preference included first choice, choice after 2 hr of feeding, percentage of leaf disc eaten after 2 hr and percentage of leaf disc eaten after 16 hr. To account for repeated measures and paired tests, we included larval ID and petri dish as random intercepts. To investigate whether altered herbivore preference could be driven by differences in performance when feeding from healthy versus infected plants, we modelled the final weight of a larva as a function of diet (either healthy leaves, OA-, TA- or UA-infected leaves), and then estimated diet-specific contrasts.

3 | RESULTS

3.1 | Effects of pathogens on plant growth and fitness

The effect of infection on plant growth differed strongly among pathogen species (Figure 2a–d; Table S3). When compared to healthy plants, OA- and TA-infected plants had longer shoots, smaller leaves and smaller overall size. SA-infected plants had shorter shoots, smaller leaves and smaller overall size. UA-infected plants tended to have shorter shoots and smaller overall size than healthy plants. Neither SA nor UA infection affected flower height. While the magnitude of these effects varied over time, the direction did not change (Figure S2; Table S3). UA- and SA-infected plants had the same number of ovules as healthy plants, but significantly fewer mature seeds (Figure 2e,f; Table S4).

3.2 | Pollinator-mediated effects of pathogens on plant fitness

The lower fitness of infected plants was not caused by reduced pollination success (Figure 3; Table S5). The hand-pollination treatment

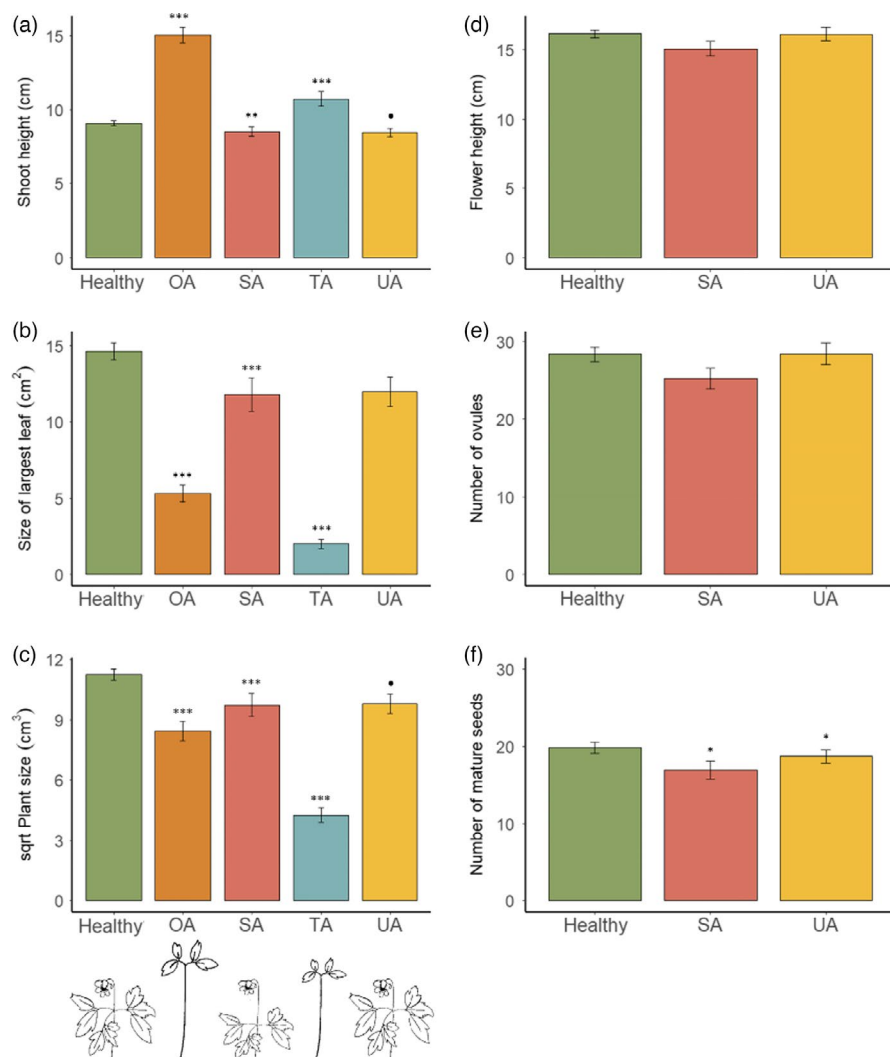


FIGURE 2 The impact of pathogens on plant growth and reproduction, in terms of (a) shoot height, (b) size of the largest leaf, (c) plant size (square root transformed), (d) flower height, (e) the number of ovules and (f) the number of mature seeds during the final week of field observations. Below the left panels is a visualization of the plant growth patterns as observed for healthy plants and infected plants. Shown are means \pm SEs. Circles and stars above the bars indicate whether there was a trend or significant difference with the healthy control, $\bullet p < 0.1$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

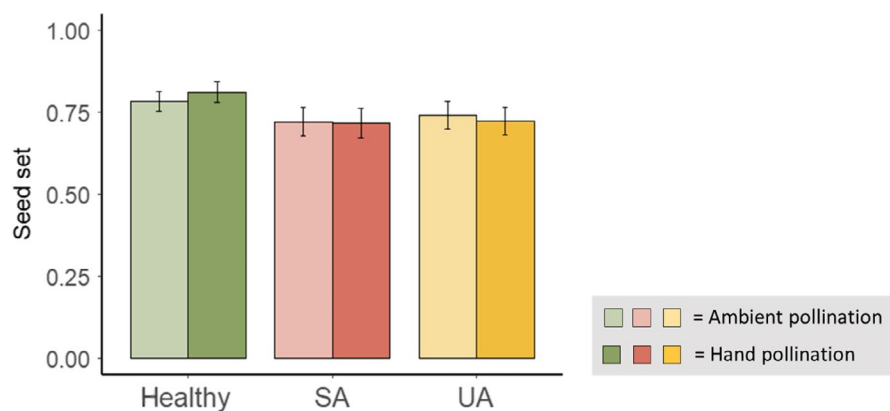


FIGURE 3 The effects of disease status (healthy, SA-infected or UA-infected) and pollination treatment (ambient pollination in light shades, hand pollination in dark shades) on seed set. Shown are least squares means \pm SEs

did not affect seed set in either infected or healthy plants (Figure 3; Table S5). Flower height and plant size did not affect seed set (Table S5).

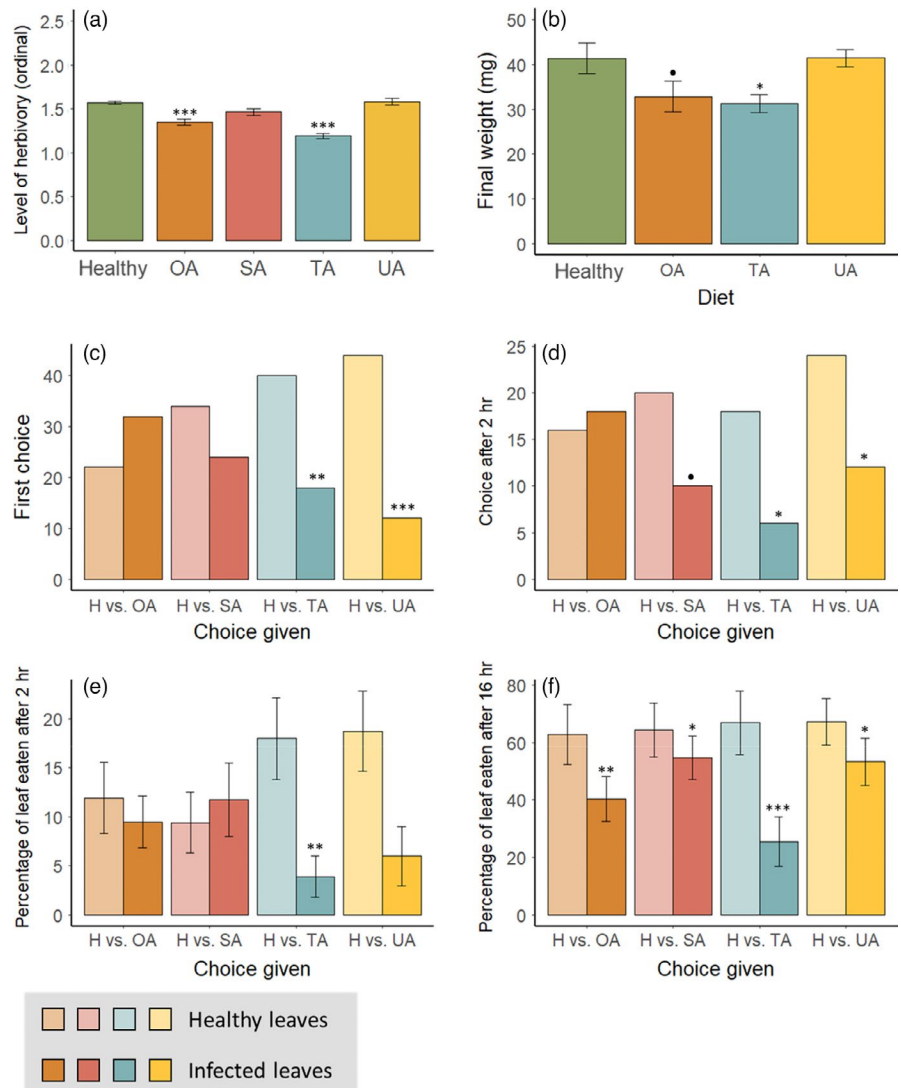
3.3 | Herbivore-mediated effects of pathogens on plant fitness

The effect of pathogen infection on herbivory strongly differed among pathogen species (Figure 4a; Table S6). SA- and

UA-infected plants had the same amount of herbivory as healthy plants, whereas OA- and TA-infected plants experienced significantly less herbivory (Figure 4a; Table S6). While plants infected by some of the pathogens had less herbivory, we detected no effects of herbivory on the number of ovules or the number of mature seeds (Table S7).

Pathogen infection influenced the preference and performance of herbivores. Herbivores preferred healthy leaves over TA- or UA-infected leaves and tended to prefer healthy leaves over SA-infected

FIGURE 4 The impact of pathogens on plant–herbivore interactions. Panel (a) shows the effect of pathogens on herbivory in the field. Panel (b) shows the results of a no-choice experiment, showing the final weights of larvae on a diet of either healthy, OA-, TA- or UA-infected leaves. Panels (c–f) show the results of a dual-choice experiment, including (c) the first choice, (d) choice after 2 hr, (e) the percentage of leaf disc eaten after 2 hr and (f) the percentage of leaf disc eaten after 16 hr, when given a choice between healthy versus OA, healthy versus SA, healthy versus TA or healthy versus UA. Light shaded bars show the healthy control leaf discs (H), and dark shaded bars show the pathogen-infected leaf discs. As not all larvae were feeding after 2 hr, the number of replicates in panel (d) is lower than in panel (c). Shown are means \pm SEs. Stars above the bars indicate whether there was a trend or a significant difference with the healthy control, $\bullet p < 0.1$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$



leaves, whereas they did not discriminate between healthy and OA-infected leaves (Figure 4c,d; Table S8a). After 2 hr, larvae had consumed a higher percentage from the healthy discs as compared to the TA-leaf discs, whereas we detected no difference in consumption of OA-, SA- or UA-leaf discs versus healthy discs (Figure 4e; Table S8a). After 16 hr, larvae had consumed more of the healthy leaf discs than the infected leaf discs for all four pathogens (Figure 4f; Table S8a).

In agreement with the preference for healthy leaf discs, final weight of larvae was significantly lower when eating from TA- or OA-infected plants versus healthy plants (Figure 4b; Table S8b). In contrast, final larval weight did not differ between the healthy and UA diet (Figure 4b; Table S8b).

4 | DISCUSSION

We investigated direct and insect-mediated effects of four fungal pathogens on the growth and fitness of the wood anemone. While negative effects of pathogens on plant growth and fitness were omnipresent, we did not detect indirect effects mediated by mutualistic

or antagonistic insects. Some of the pathogens influenced herbivore preference and performance, which likely contributed to the differences in herbivory between infected and healthy plants in the field. Our study shows that effects of pathogens on plants are direct rather than insect-mediated, and that the impacts of infection on plant growth and fitness are highly variable among pathogen species. Moreover, pathogen species differed in their effects on herbivore preference and performance, as well as herbivore damage in the field, but this did not translate into effects on plant fitness.

4.1 | Direct effects of pathogens on plant growth and fitness

We found overall negative effects of pathogens on plant growth, sexual reproduction and fitness, though the magnitude of effects differed among pathogen species. The growth pattern of infected plants differed strongly among pathogens: OA- and TA-infected plants had elongated shoots but very small leaves, whereas SA-infected plants had shorter shoots and smaller leaves than healthy

plants. We did not detect any differences in flower height for SA- or UA-infected plants compared to healthy plants. Our findings on the divergent growth patterns of anemones infected by the systemic rusts OA and TA are consistent with previous observations within this system (García-Guzmán & Wennström, 2001). Shoot elongation of plants infected by systemic rusts has been reported earlier, such as for *Lactuca sibirica* systemically infected by the rust *Puccinia minussensis*, and may be an adaptive strategy of the fungus to promote spore dispersal (García-Guzmán & Wennström, 2001; Wennström & Ericson, 1991). On the other hand, the smaller size of SA-infected plants, as well as the tendency for smaller size of UA-infected plants, may be related to resource limitation (Huot et al., 2014). Thus, while we found a general trend for negative effects of pathogens on plant growth, we also identified striking differences among pathogens in how they affected plant growth patterns.

In line with our expectations, infected plants had lower fitness. While SA- and UA-infected plants had the same number of ovules as healthy plants, they produced fewer mature seeds. Maternal investment into reproductive traits, including flower production, ovule production and seed maturation, can be adjusted over time in response to available resources—an adaptive strategy of the plant to maximize fitness when future availability of resources is unpredictable (Lloyd, 1980; Sultan, 2000). Pathogens can reduce the resources available to their hosts by extracting nutrients, reducing photosynthetic area and inducing costly defence pathways (Huot et al., 2014; Lovett-Doust & Lovett-Doust, 1988). Fewer resources for infected plants could have led to discontinued maternal investment into seed maturation. Our study also showed that pathogens influenced the sexual reproductive strategies of host plants. Seed production of wood anemone flowers was lower upon infection by SA or UA, while for OA- and TA-infected plants flower production was inhibited (García-Guzmán & Wennström, 2001). All pathogens thus had negative impacts on plant fitness, albeit to a different degree and due to different mechanisms. This finding is in line with the notion that pathogens differ in their impacts on the resource budget, and induce distinct chemical and physiological reactions in their host (Jarosz & Davelos, 1995). The two pathogen species that were phylogenetically most closely related (OA and TA), their genera being sister groups (Maier et al., 2011), had most similar effects. While based on very few species, these findings are consistent with that genetically more closely related species have more similar traits, and thus have more similar impacts on host plant growth and fitness. The similar impact on plant growth and fitness might then reflect the fact that they manipulate the hormonal pathways of anemone in similar ways, resulting in inhibited flower production in infected plants. Future studies on multiple pathogens infecting other host plant species will be essential to determine how variation in plant responses to disease depends on host plant species versus pathogen species. To elucidate whether observed relationships between infection and fitness are indeed causal, one might experimentally infect healthy plants.

4.2 | Pollinator-mediated effects of pathogens on plant fitness

Contrary to our expectations, we did not detect pollinator-mediated effects of pathogens on plant fitness. In order for pollinator-mediated effects to be of evolutionary importance for plants, it is required that pathogens influence cues that affect pollinator visitation, and that altered pollinator visitation affects plant fitness. There is some evidence that pathogens can influence plant cues that are potentially relevant to pollinators (Groen et al., 2016; Himeno et al., 2011; Stevenson et al., 2017), but we lack studies that have explored related fitness impacts in natural systems (Table S1). While our study mainly focussed on experimentally assessing the effects of a diverse set of pathogens on plant fitness mediated by pollinators, and not on exploring a diverse set of traits potentially mediating such interactions, we did measure flower height, which could potentially serve as a visual cue for pollinators (Ehrlén et al., 2002). However, we detected no effect of infection on flower height, and flower height also did not influence pollination success. Concerning olfactory cues, one previous study, conducted under controlled laboratory conditions, found increased pollinator preference for virus-infected tomato plants due to altered volatile emissions (Groen et al., 2016). As viruses commonly use insects as vectors for dispersal, the attraction of insects to the host plant can be an adaptive strategy of the virus (Chesnaïs et al., 2019; Mauck et al., 2016). Thus, pathogen-induced effects on pollinators could either arise as a side effect of pathogen exploitation of host plant resources (Jarosz & Davelos, 1995) or as changes induced by the pathogen to promote dispersal (Roy, 1993). Although several changes induced by pathogens might potentially affect pollinator behaviour, the lack of documented fitness effects makes it hard to assess the evolutionary significance of this pathway. While our finding of no pollinator-mediated effects of pathogen infection on plant fitness could be caused by the absence of pathogen-induced effects on pollinators, it is also possible that pathogen infection actually led to decreased pollinator visitation rates, but that pollination levels were still sufficient to maintain high seed set (Zimmerman & Aide, 1989). This notion is supported by the results of our hand-pollination experiment, where hand-pollinated flowers had a seed set similar to flowers with ambient pollination only. Although we did not find any evidence of pollinator-mediated effects of the four major fungal pathogens on the fitness of wood anemone, it would be interesting for future studies to explore if pathogen-induced changes in plant morphology might lead to pollinator-mediated effects on plant fitness under conditions with a lower availability of pollinators.

4.3 | Herbivore-mediated effects of pathogens on plant fitness

Our field observations showed that OA- and TA-infected plants suffered from less herbivory than healthy plants, whereas SA- or UA-infected plants experienced similar levels. As OA and TA showed strongly altered growth patterns compared to healthy, UA- and SA-infected plants, this suggests that visual cues may have affected

host plant selection of herbivores in the field. In line with the field observations, our feeding trials showed that larvae had lower preference for leaf tissues of infected plants, and lower performance on OA- and TA-infected plants. This suggests a correlation between herbivore preference and performance, indicating that the sawfly larvae *Monophadnus pallescens* have evolved a preference for plants on which they perform best (Gripenberg et al., 2010). Our findings are also in line with a meta-analysis by Fernandez-Conradi et al. (2018), which showed that fungal infections reduce herbivore preference and performance. Still, we also found variation in herbivore responses depending on the pathogen: In contrast to the negative effects of OA and TA infection on both larval preference and performance, UA infection reduced preference but not performance. As lesions on UA-infected plants are localized, larvae could possibly avoid feeding on infected tissues. In case effects of infection on nutritional value are restricted to lesions, this strategy could limit impacts on larval performance (Simon & Hilker, 2003). On the other hand, larvae that feed on healthy tissues of infected plants may still be affected by plant responses elicited by the pathogen (Rostás et al., 2003). Larvae feeding on OA- and TA-infected leaves could potentially have ingested less plant tissues to avoid feeding on lesions, while larvae on a UA diet could consume more plant tissues without ingesting spores. Interestingly, in parallel to the effects on plant performance, the two pathogens that were most closely related (OA and TA) were most similar with regard to herbivory, herbivore preference and herbivore performance. The observed differences in the effects of pathogens on herbivores exemplify that pathogen identity can influence the distribution and abundance of plant-associated herbivores, thus having ecological and evolutionary consequences for plant-associated insect communities (Biere & Tack, 2013; Busby et al., 2015; Tack & Dicke, 2013).

Contrary to our expectations, and despite the effects of pathogens on herbivores, we did not find herbivore-mediated effects of pathogens on plant fitness. Herbivory was only reduced on plants infected by pathogens that inhibited flowering (OA and TA) and not on infected plants with flowers (SA- and UA-infected). Potential positive effects of decreased herbivory on seed production could evidently not be detected for plants without flowers. Another possible explanation for absent herbivore-mediated effects on plant fitness is that the wood anemone is a rhizomatous species. Hence, attacker damage during one season might lead to decreased allocation of resources to belowground storage, and impacts on growth and fitness become apparent only in subsequent years (Larcher, 1995; Suzuki & Stuefer, 1999). Thus, an interesting future research avenue would be to explore herbivore-mediated effects on plant fitness across multiple years, in order to gain a comprehensive understanding about the indirect and long-term impacts of pathogens on their host plant.

5 | CONCLUSIONS

Our study advances two main generalizations. First, we demonstrated that the impacts of pathogens on plant growth and fitness

were generally negative, suggesting that pathogens constitute important ecological factors and selective agents. Second, our study showed that pathogens affect their host plant directly rather than via insect-mediated mechanisms. Dominance of direct effects was observed in spite of effects of pathogens on traits potentially affecting herbivores and pollinators, and in spite of observed negative effects of some pathogens on herbivory, herbivore preference and herbivore performance. Understanding the ecological and evolutionary impacts of pathogens on plant communities and plant-associated species is important for biodiversity conservation, given the expected changes in the abundance and distribution of pathogens and spread of invasive pathogens with climate change and other anthropogenic activities (Barnes et al., 2014; Ramsfield et al., 2016).

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AUTHORS' CONTRIBUTIONS

L.J.A.v.D., A.J.M.T. and J.E. designed the research; L.J.A.v.D. conducted the field and experimental work, analysed the data and wrote the manuscript, with contributions from all authors.

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DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.wh70rxwn9> (van Dijk et al., 2021).

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SUPPORTING INFORMATION

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