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Srisakrapikoop, U., Pirie, T. J. and Fellowes, M. D. E. (2021) Aphids show interspecific and intraspecific variation in life history responses to host plant infection by the fungal pathogen *Botrytis cinerea*. *Entomological Science*. ISSN 1479-8298 doi: <https://doi.org/10.1111/ens.12476> Available at <http://centaur.reading.ac.uk/98744/>

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To link to this article DOI: <http://dx.doi.org/10.1111/ens.12476>

Publisher: Wiley

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## SHORT COMMUNICATION

# Aphids show interspecific and intraspecific variation in life history responses to host plant infection by the fungal pathogen *Botrytis cinerea*

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## Abstract

The life histories of insect herbivores are affected by variation in host plant quality, with poor quality typically being associated with reduced herbivore fecundity, size and longevity. Plant pathogens are ubiquitous in nature and can alter host plant quality as experienced by insect herbivores. We asked how host plant infection by the widespread and economically important fungal pathogen *Botrytis cinerea* affected the life history traits of two aphid species. We found that the life history traits of the black bean aphid *Aphis fabae* were negatively affected by being reared on infected host plants, showing reduced fecundity, population growth rate, size, off-plant survival time and development rate. In contrast, we found that pea aphids *Acyrtosiphon pisum* benefitted from being reared on infected plants, and that the degree of benefit varied between pea aphid clonal lines. This work suggests that the ecological and economic consequences of plant pathogen infection on the dynamics of aphid pests could be difficult to predict.

**Key words:** black bean aphid, clonal variation, gray mold, pea aphid, plant pathogen, plant-mediated indirect effects.

*Botrytis* spp. are globally important fungal plant pathogens, causing disease in >1400 plant species (Elad *et al.* 2016), including many economically important crops (Elad *et al.* 2004). *Botrytis cinerea* is an aggressive necrotrophic fungus that destroys host plants with necrotic lesions (Shaw *et al.* 2016) and is perhaps the most notorious species of this genus, causing dramatic losses in both pre- and post-harvest crops (Dean *et al.* 2012). *Botrytis cinerea* has been ranked as the second most important fungal pathogen in terms of its scientific and economic value (Dean *et al.* 2012).

Aphids are among the most important crop pests in temperate regions (van Emden & Harrington 2007), causing both direct damage to host plants and indirect damage by acting as vectors of plant viruses and by the production of honeydew, which can result in fungal infection and reduce photosynthesis (van

Emden 2013). Aphids show both between and within species variation in their life history responses to environmental factors such as host plant quality (Service 1984; Stacey *et al.* 2002a), temperature (Stacey *et al.* 2002b; Stacey *et al.* 2003), and crowding (Hazell *et al.* 2005). Such variation will have economic and ecological consequences, affecting which species or genotypes are likely to benefit from such changes (Thompson 1988; Bolnick *et al.* 2011; Des Roches *et al.* 2018).

Fungal plant pathogen infection can alter host plant quality as experienced by herbivores by inducing biochemical defense responses inside the host plant. These biochemical responses can also negatively affect herbivorous insects (Fernandez-Conradi *et al.* 2018; Ederli *et al.* 2021) and indeed could also affect species at higher trophic levels (Nghah *et al.* 2018; Srisakrapikoop *et al.* 2020). Nevertheless, the effect of plant pathogen infection might also benefit some insect herbivores (Tack & Dicke 2013), and herbivores could differ in their responses to host plants infected by different plant pathogens (e.g. positive effects found for *Aphis fabae* feeding on *Vicia faba* infected by *Uromyces viciae-*

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Received 4 January 2021; accepted 23 April 2021.

*fabae*, but negative effects are seen when *V. faba* is infected by *B. cinerea*; Al-Naemi & Hatcher 2013).

What is not clear is whether different species of aphid or aphid genotypes differ in the life history consequences of feeding on the same host plant species infected by the same plant pathogen. We addressed these questions using two aphid species, the black bean aphid *Aphis fabae* and the pea aphid *Acyrtosiphon pisum*, and three clones of the latter species, asking whether host plant infection status influenced the size, fecundity, maturation time and off-plant survival of the study aphids.

*Botrytis cinerea* Pers.: Fr (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel) was cultured on malt extract agar and incubated at 20°C under conditions of 12 h UV light : 12 h dark (LD 12:12) to encourage the fungus to produce spores.

The host plants, *Vicia faba* L. (Fabaceae, cv. Sutton dwarf), were individually grown in 1 L pots with peat compost (Clover®, London, UK). When the plants had five true leaves, they were divided into two treatment groups. Plants in the infected group were treated with a 0.1 mL suspension of 1-month old *B. cinerea* (10<sup>6</sup>–conidia/mL) on the adaxial surfaces of the leaves using a paint brush. Uninfected plants were treated with distilled water in a similar manner. Plants were then kept individually in a sealed polythene bag at 20°C for 48 h to encourage spore germination.

A single black bean aphid *Ap. fabae* Scop. (Hemiptera: Aphididae) was collected from opium poppy *Papaver somniferum* L. (Papaveraceae), and three pea aphid *Ac. pisum* Harris (Hemiptera: Aphididae) clones were collected from bird's-foot trefoil *Lotus corniculatus* L. (Fabaceae), from three widely separated locations, all in the Whiteknights campus of the University of Reading, UK. The two aphid species were identified and confirmed following Blackman and Eastop (2000). Aphid cultures were maintained as a monoclonal culture in separate insect cages and provided with either uninfected or infected *V. faba* plants for more than three generations before the experiments started to avoid confounding maternal effects. All work was carried out in a controlled environment room at 20°C, LD 16:8, 60% RH.

For the black bean aphid experiment, treatments comprised of five uninfected or five infected plants, each of which held eight aphids. Each aphid was confined individually in a clip cage (20 mm in diameter; Noble 1958) attached to individual leaflets (total 40 aphids/infection status; aphids were transferred from culture plants of the same infection status to avoid confounding maternal effects). Aphids were left to produce nymphs for 24 h, then all apart from one nymph were removed, which was allowed to grow to maturity and produce offspring.

Time to maturity was recorded and the number of offspring produced was then recorded every second day for 10 days. During each visit nymphs were removed to prevent competition. The intrinsic rate of increase ( $r_m$ ) was calculated from the formula  $r_m = (c \ln [Md]) / D$ , where  $c$  is a constant (0.738),  $Md$  is the number of offspring produced by the adult aphid in the  $D$  days of reproduction (Wyatt & White 1977).

Separately, 80 7-day-old aphids (40 from each treatment) were randomly selected from cultures and transferred into individual Petri dishes without food or water and monitored every 8 h until death to yield off-plant survival time. Another 80 7-day-old aphids (40 from each treatment) were used to measure hind tibia length (Nicol & Mackauer 1999) under a high-performance stereomicroscope (Leica MZ9.5; Heerbrugg, Switzerland).

For the pea aphid experiment, 10 adult apterous pea aphids from each of the three clones were randomly selected from each of the base culture colonies feeding on uninfected and infected plants (60 aphids in total). Each aphid was placed into an individual clip cage (40 mm in diameter) directly onto an individual plant of the same colony infection status. Fecundity and intrinsic rate of increase were recorded in the same manner as described above, except the number of offspring were recorded every other day for 14 days.

In addition, aphid off-plant survival time, hind tibia size, days to maturity and the intrinsic rate of increase were also recorded again in a similar manner as for *Ap. fabae*. A total of 180 nymphs (30 from each clone and treatment) were allowed to grow for 7 days, reaching the 4th instar stage. When the individuals were transferred into a Petri dish without food or water, they were monitored every 12 h until death. Forty 7-day-old aphids (from each clone and treatment) were used to measure hind tibia length under a high-performance stereomicroscope (MZ9.5; Leica).

All statistical analyses were carried out on R 4.0.3 (R Core Team 2020). For the *Ap. fabae* experiment, as the data are not normally distributed, Wilcoxon rank sum tests were used to test for differences in hind tibia length and off-plant survival time between infected and uninfected plants. Initial examination of the data showed that the effect of nested data could be ignored as the variances resulting from different plants were very close to zero, and then the intrinsic rate of increase, fecundity and maturation time data could also be analyzed by using Wilcoxon rank sum tests.

In the *Ac. pisum* experiment the hind tibia length, off-plant survival time, maturation time and intrinsic rate of increase data were analyzed using ANOVA of aligned rank transformed using the *ARTool* package

(Wobbrock *et al.* 2011) as data were not normally distributed. Fecundity data were analyzed by ANOVA using the *car* package (Fox & Weisberg 2019). Post hoc analyses with Tukey tests were analyzed using the *emmeans* package (Lenth 2019) or with the Mann–Whitney *U*-test to examine clonal variation within the infection status group.

*Aphis fabae* feeding on plants infected by *B. cinerea* had significantly shorter hind tibia length ( $W = 1148$ ,  $n_1 = 40$ ,  $n_2 = 39$ ,  $P < 0.001$ ; Fig. 1a), reduced off-plant survival time ( $W = 509$ ,  $n_1 = 40$ ,  $n_2 = 39$ ,  $P = 0.007$ ; Fig. 1b), slower development rates ( $W = 402$ ,  $n_1 = 38$ ,  $n_2 = 33$ ,  $P = 0.016$ ; Fig. 1c), reduced intrinsic rate of increase ( $W = 880$ ,  $n_1 = 38$ ,  $n_2 = 33$ ,  $P = 0.004$ ; Fig. 1d) and lower fecundity ( $W = 880$ ,  $n_1 = 38$ ,  $n_2 = 33$ ,  $P = 0.004$ ; Fig. 1e).

Pea aphids feeding on plants infected by *B. cinerea* had significantly longer hind tibia lengths than those on uninfected plants ( $F_{1,226} = 49.17$ ,  $P < 0.001$ ; Fig. 2a). There was no overall effect of aphid clone on hind tibia length ( $F_{2,226} = 2.01$ ,  $P = 0.137$ ), but the interaction term was significant ( $F_{2,226} = 3.87$ ,  $P = 0.022$ ). There was no effect of host plant infection status on off-plant survival time ( $F_{1,173} = 1.35$ ,  $P = 0.246$ ), but this differed between clones ( $F_{2,173} = 61.49$ ,  $P < 0.001$ ; Fig. 2b), and there was a significant interaction effect of clone and host plant infection status on off-plant survival ( $F_{2,173} = 4.68$ ,  $P = 0.011$ ). Time to maturity differed among clones ( $F_{2,54} = 34.82$ ,  $P < 0.001$ ; Fig. 2c), and differed between plant infection status ( $F_{1,54} = 10.82$ ,  $P = 0.002$ ). The interaction term was significant ( $F_{2,54} = 13.73$ ,  $P < 0.001$ ). The intrinsic rate of increase differed between pea aphid clones ( $F_{2,38} = 4.09$ ,  $P = 0.025$ ; Fig. 2d) and was significantly higher on infected plants ( $F_{1,38} = 34.95$ ,  $P < 0.001$ ). The interaction term was significant ( $F_{2,38} = 7.20$ ,  $P = 0.002$ ). The fecundity of aphids feeding on infected plants was significantly higher than those feeding on uninfected plants ( $F_{1,40} = 13.95$ ,  $P < 0.001$ ; Fig. 2e), but there was no clone ( $F_{2,40} = 1.30$ ,  $P = 0.283$ ) or interaction effect ( $F_{2,38} = 1.04$ ,  $P = 0.364$ ).

There is increasing evidence showing that plant pathogens play important roles in determining the interaction between insects and their host plants, and indeed these effects can ramify through communities (Grunseich *et al.* 2020; Srisakrapikoop *et al.* 2020). In this study, we find that two aphid species respond in different directions to the same plant pathogen–host system. Host plant infection by *B. cinerea* caused negative indirect effects on *Ap. fabae* for all measured parameters, whereas infection caused positive indirect effects on *Ac. pisum*, and here the magnitude of these effects differed between aphid clones. The indirect

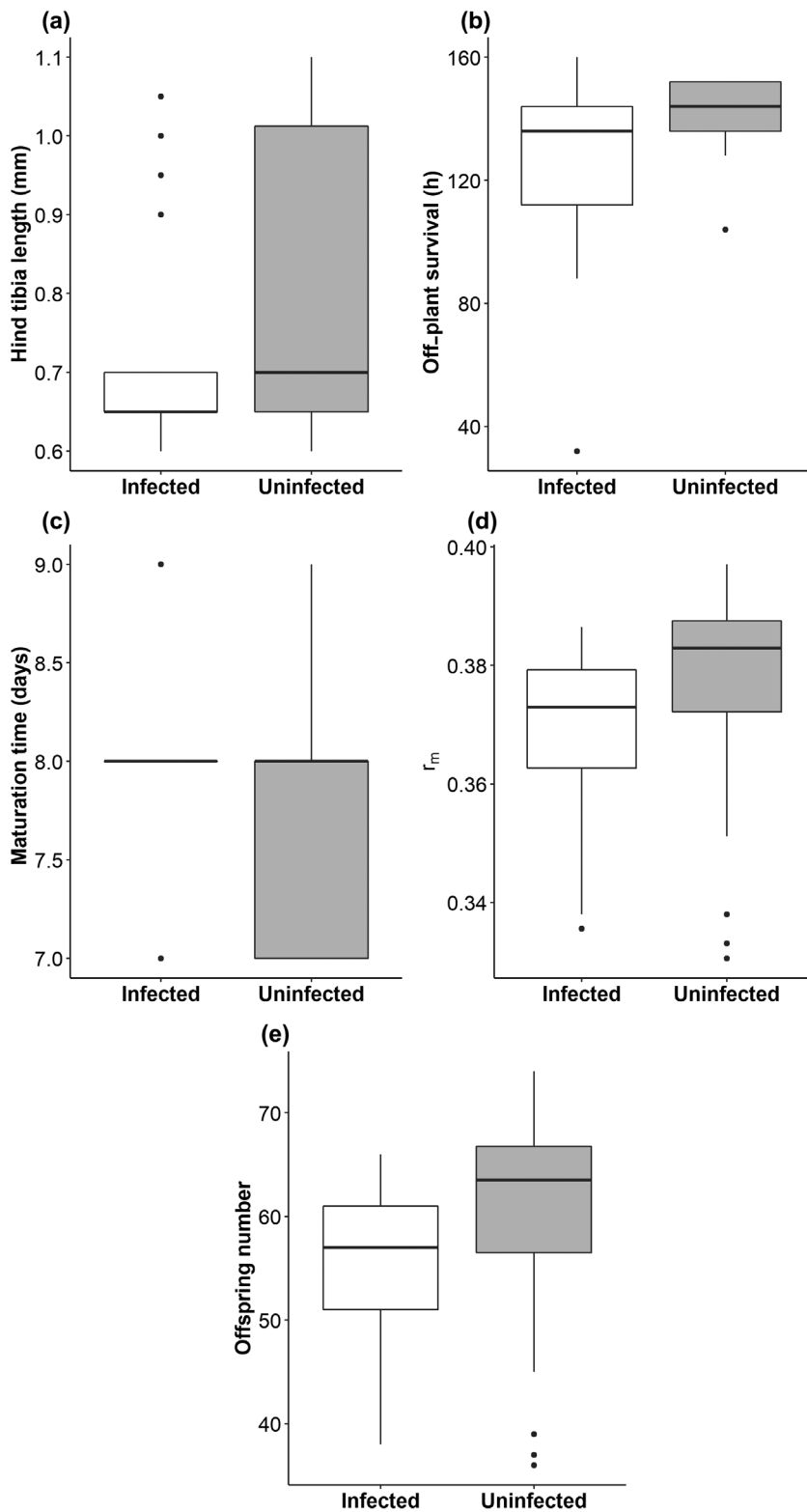
effects of plant pathogen infection therefore differ both between and within aphid species.

*Aphis fabae* feeding on uninfected plants performed better than those feeding on infected plants in all measured parameters, which is consistent with a previous study with a similar system (Al-Naemi & Hatcher 2013). *Acyrtosiphon pisum* expressed the reverse pattern, benefitting from feeding on infected plants, and these effects were consistent, but differed in magnitude, across clones. The mechanisms underpinning the between species differences are not clear, given that both are generalist aphid species, which feed in a similar manner, and the host plant–pathogen treatment was controlled. The ultimate cause of this variation is worthy of more detailed study, as it suggests that the population dynamics of different aphid species might be differentially affected by pathogen infection.

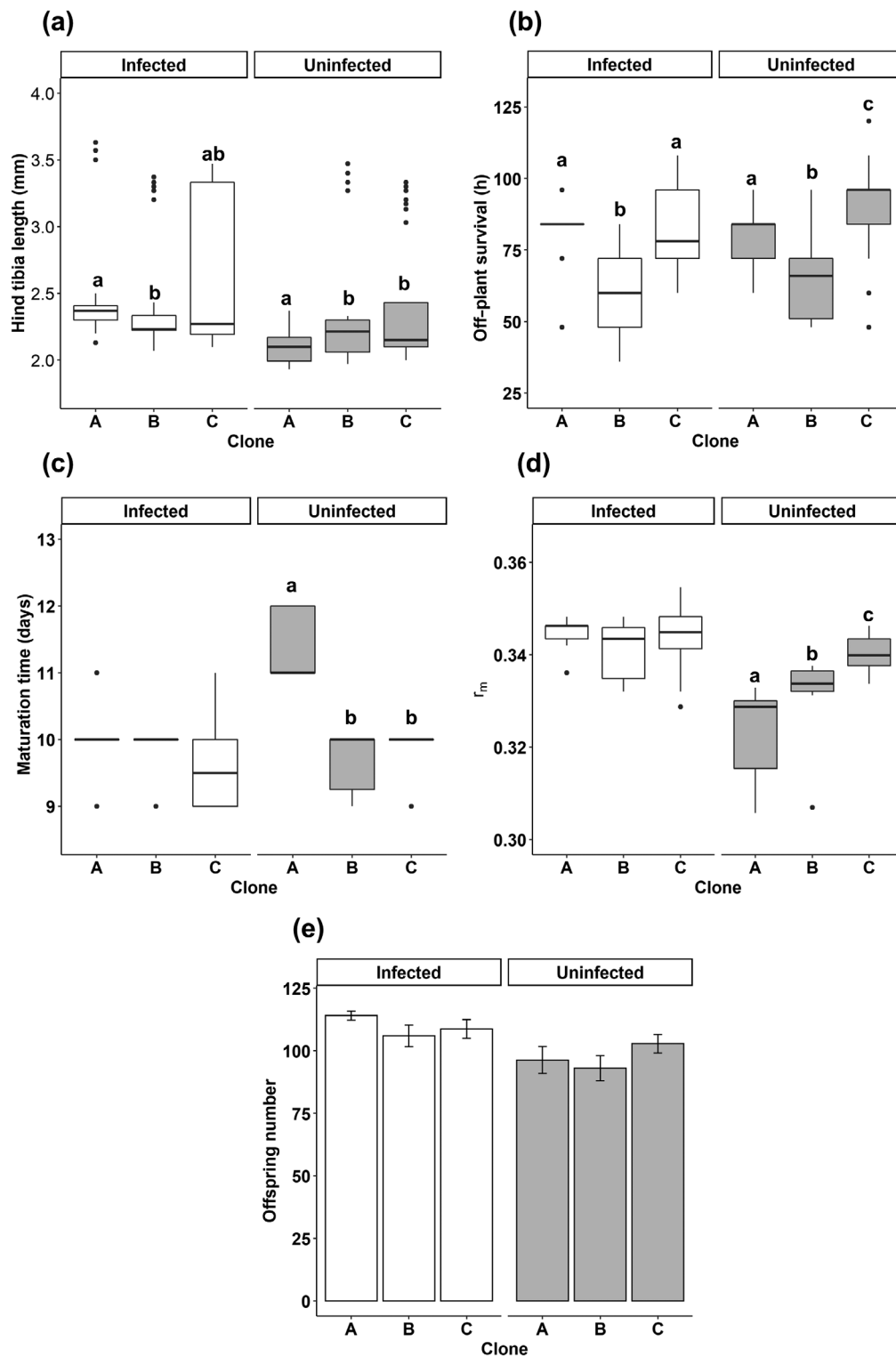
Although the causes of the differences in response are unclear, we note that the indirect effects of *B. cinerea* on aphid life histories could be transmitted in two (non-independent) pathways, either through a change in host nutrition and/or in host defense. The former is caused by a change in nitrogen content, which is decreased by fungal infection (Dulermo *et al.* 2009; Al-Naemi & Hatcher 2013), and the latter is induced resistance, where the plant responds to infection through two signaling routes, the salicylic acid (SA) and jasmonic acid (JA) pathways (Pieterse *et al.* 2014). The SA pathway is usually used by plants to respond to sucking/piercing herbivores (including aphids), whereas the JA pathway is upregulated in response to necrotrophic pathogens and chewing herbivores (Pieterse *et al.* 2014; Stout 2014), and these pathways are considered to trade-off against each other (Spoel *et al.* 2003; Brooks *et al.* 2005).

In this study, the plants had been first infected by *B. cinerea* before aphids were introduced, so it is likely that the JA pathway was triggered, suppressing the SA pathway (Leon-Reyes *et al.* 2010). Theoretically, aphids should benefit from this, but here *Ap. fabae* showed reduced performance when feeding on infected plants, perhaps because aphids are not only affected by outcomes of suppressing the SA pathway (Thaler *et al.* 2010), but also by the elicited JA pathway (Thaler *et al.* 2001; Goggin & Cooper 2005). Aphid species could therefore differ in their responses to changes in nutrition and plant defenses. In contrast, variation in the strength of within species responses between aphid clones is expected, given the importance of both genetic and endosymbiotic factors (Stacey *et al.* 2003; Hazell & Fellowes 2009; Heyworth *et al.* 2020).

This work is of considerable applied interest, as we show that the effects of infection by an economically very important plant pathogen on the life histories of two



**Figure 1** Medians, interquartiles and range of (a) hind tibia length, (b) off-plant survival time, (c) maturation time, (d) intrinsic rate of increase and (e) fecundity of *Aphis fabae* feeding on *Botrytis cinerea* infected and uninfected host plants.



**Figure 2** Medians, interquartiles and range of (a) hind tibia length, (b) off-plant survival time, (c) maturation time, (d) intrinsic rate of increase and (e) mean  $\pm$  SE fecundity for three *Acyrtosiphon pisum* clones (A, B and C) feeding on *Botrytis cinerea* infected and uninfected host plants. The letters above the bars show traits where a significant difference ( $P < 0.05$ ) was found within infection status group.

related and also economically important aphid pest species differ. Feeding on *B. cinerea*-infected host plants benefits pea aphids (albeit the strength of this varies across clones), whereas black bean aphids are detrimentally affected by infected host plants. The cause of this difference between species is unclear. This could be the result of differences in the direct physiological effects of host plant quality on the developing aphids, or an indirect effect mediated by differences in other factors, such as the composition of the aphid's endosymbiont community. The links between plant pathogens and insect herbivores are rarely simple; elucidating the mechanisms that result in such unexpected contrasting effects would be of considerable value.

## ACKNOWLEDGMENTS

We would like to thank the Development and Promotion of Science and Technology Talents Project (DPST), Royal Government of Thailand scholarship (to US).

## REFERENCES

- Al-Naemi F, Hatcher PE (2013) Contrasting effects of necrotrophic and biotrophic plant pathogens on the aphid *Aphis fabae*. *Entomologia Experimentalis et Applicata* **148**, 234–245.
- Blackman RL, Eastop VF (2000) *Aphids on the World's Crops: An Identification and Information Guide*, 2nd edn. John Wiley & Sons, Somerset.
- Bolnick DI, Amarasekare P, Araújo MS *et al.* (2011) Why intraspecific trait variation matters in community ecology. *Trends in Ecology and Evolution* **26**, 183–192.
- Brooks DM, Bender CL, Kunkel BN (2005) The *Pseudomonas syringae* phytotoxin coronatine promotes virulence by overcoming salicylic acid-dependent defences in *Arabidopsis thaliana*. *Molecular Plant Pathology* **6**, 629–639.
- Dean R, van Kan JAL, Pretorius ZA *et al.* (2012) The top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology* **13**, 414–430.
- Des Roches S, Post DM, Turley NE *et al.* (2018) The ecological importance of intraspecific variation. *Nature Ecology and Evolution* **2**, 57–64.
- Dulermo T, Bligny R, Gout E, Cotton P (2009) Amino acid changes during sunflower infection by the necrotrophic fungus *B. cinerea*. *Plant Signaling & Behavior* **4**, 859–861.
- Ederli L, Salerno G, Quaglia M (2021) In the tripartite combination *Botrytis cinerea* – *Arabidopsis* – *Eurydema oleracea*, the fungal pathogen alters the plant–insect interaction via jasmonic acid signalling activation and inducible plant-emitted volatiles. *Journal of Plant Research* **134**, 523–533.
- Elad Y, Williamson B, Tudzynski P, Delen N (2004) *Botrytis* spp. and diseases they cause in agricultural systems – an introduction. In: Elad Y, Williamson B, Tudzynski P, Delen N (eds) *Botrytis: Biology, Pathology and Control*. Kluwer Academic, Dordrecht, 2.
- Elad Y, Pertot I, Cotes Prado AM, Stewart A (2016) Plant hosts of *Botrytis* spp. In: Fillinger S, Elad Y (eds) *Botrytis – The Fungus, the Pathogen and its Management in Agricultural Systems*. Springer, Cham, 413.
- Fernandez-Conradi P, Jactel H, Robin C, Tack AJM, Castagnyrol B (2018) Fungi reduce preference and performance of insect herbivores on challenged plants. *Ecology* **99**, 300–311.
- Fox J, Weisberg S (2019) *An R Companion to Applied Regression*. Sage, Thousand Oaks.
- Goggin FL, Cooper WR (2005) Effects of jasmonate-induced defenses in tomato on the potato aphid, *Macrosiphum euphorbiae*. *Entomologia Experimentalis et Applicata* **115**, 107–115.
- Grunseich JM, Thompson MN, Aguirre NM, Helms AM (2020) The role of plant-associated microbes in mediating host-plant selection by insect herbivores. *Plants* **9**, 1–23.
- Hazell SP, Fellowes MDE (2009) Intra-specific variation affects the structure of the natural enemy assemblage attacking pea aphid colonies. *Ecological Entomology* **34**, 34–42.
- Hazell SP, Gwynn DM, Ceccarelli S, Fellowes MDE (2005) Competition and dispersal in the pea aphid: clonal variation and correlations across traits. *Ecological Entomology* **30**, 293–298.
- Heyworth ER, Smee MR, Ferrari J (2020) Aphid facultative symbionts aid recovery of their obligate symbiont and their host after heat stress. *Frontiers in Ecology and Evolution* **8**, 56.
- Lenth R (2019) emmeans: estimated marginal means, aka least-squares means. Available from URL: <https://cran.r-project.org/package=emmeans>
- Leon-Reyes A, Du Y, Koornneef A *et al.* (2010) Ethylene signaling renders the jasmonate response of *Arabidopsis* insensitive to future suppression by salicylic acid. *Molecular Plant–Microbe Interactions* **23**, 187–197.
- Ngah N, Thomas RL, Shaw MW, Fellowes MDE (2018) Asymptomatic host plant infection by the widespread pathogen *Botrytis cinerea* alters the life histories, behaviors, and interactions of an aphid and its natural enemies. *Insects* **9**, 80.
- Nicol CMY, Mackauer M (1999) The scaling of body size and mass in a host–parasitoid association: influence of host species and stage. *Entomologia Experimentalis et Applicata* **90**, 83–92.
- Noble MD (1958) A simplified clip cage for aphid investigations. *The Canadian Entomologist* **90**, 760.
- Pieterse CMJ, Zamioudis C, Van der Does D, Van Wees SCM (2014) Signalling networks involved in induced resistance. In: Walters DR, Newton AC, Lyon GD (eds) *Induced Resistance for Plant Defense: A Sustainable Approach to Crop Protection*. John Wiley & Sons, Chichester, 59–63.
- R Core Team (2020) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical



- Computing, Vienna. Available from URL: [https:// www.R-project.org/](https://www.R-project.org/).
- Service P (1984) Genotypic interactions in an aphid–host plant relationship: *Uroleucon rudbeckiae* and *Rudbeckia laciniata*. *Oecologia* **61**, 271–276.
- Shaw MW, Emmanuel CJ, Emilda D *et al.* (2016) Analysis of cryptic, systemic *Botrytis* infections in symptomless hosts. *Frontiers in Plant Science* **7**, 625.
- Spoel SH, Koornneef A, Claessens SMC *et al.* (2003) NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. *The Plant Cell* **15**, 760–770.
- Srisakrapikoop U, Pirie TJ, Fellowes MDE (2020) Meta-analysis suggests differing indirect effects of viral, bacterial, and fungal plant pathogens on the natural enemies of insect herbivores. *Insects* **11**, 765.
- Stacey DA, Fellowes MDE (2002a) Influence of elevated CO<sub>2</sub> on interspecific interactions at higher trophic levels. *Global Change Biology* **8**, 668–678.
- Stacey DA, Fellowes MDE (2002b) Influence of temperature on pea aphid *Acyrtosiphon pisum* (Hemiptera: Aphididae) resistance to natural enemy attack. *Bulletin of Entomological Research* **92**, 351–357.
- Stacey DA, Thomas MB, Blanford S, Pell JK, Pugh C, Fellowes MDE (2003) Genotype and temperature influence pea aphid resistance to a fungal entomopathogen. *Physiological Entomology* **28**, 75–81.
- Stout MJ (2014) Types and mechanisms of rapidly induced plant resistance to herbivorous arthropods. In: Walters DR, Newton AC, Lyon GD (eds) *Induced Resistance for Plant Defense: A Sustainable Approach to Crop Protection*. John Wiley & Sons, Chichester, 83–84.
- Tack AJM, Dicke M (2013) Plant pathogens structure arthropod communities across multiple spatial and temporal scales. *Functional Ecology* **27**, 633–645.
- Thaler JS, Stout MJ, Karban R, Duffey SS (2001) Jasmonate-mediated induced plant resistance affects a community of herbivores. *Ecological Entomology* **26**, 312–324.
- Thaler JS, Agrawal AA, Rayko H (2010) Salicylate-mediated interactions between pathogens and herbivores. *Ecology* **91**, 1075–1082.
- Thompson JN (1988) Variation in interspecific interactions. *Annual Review of Ecology and Systematics* **19**, 65–87.
- van Emden HF (2013) *Handbook of Agricultural Entomology*. Markono Print Media, Singapore.
- van Emden HF, Harrington R (2007) *Aphids as Crop Pests*. CABI, Wallingford.
- Wobbrock JO, Findlater L, Gergle D, Higgins JJ (2011) The aligned rank transform for nonparametric factorial analyses using only ANOVA procedures. In *Proceedings of the SIGCHI Conference on Human Factors in Computing Systems (CHI '11)*, New York, NY: Association for Computing Machinery, 143–146.
- Wyatt IJ, White PF (1977) Simple estimation of increase rates for aphids and tetranychid mites. *Journal of Applied Ecology* **14**, 757–766.