RESEARCH ARTICLE

Annals of Applied Biology
An international journal of the aab

WILEY

The effects of drought stress and type of fertiliser on generalist and specialist herbivores and their natural enemies

Muhammad Shehzad[1] | Asim Gulzar[1] | Joanna T. Staley[2] | Muhammad Tariq[1]

[1]Department of Entomology, PMAS-Arid Agriculture University Rawalpindi, Rawalpindi, Pakistan

[2]UK Centre for Ecology and Hydrology, Oxfordshire, UK

Correspondence
Muhammad Tariq, Department of Entomology, PMAS-Arid Agriculture University Rawalpindi, Shamsabad, Muree Road, Rawalpindi, Pakistan.
Email: mtariq@uaar.edu.pk

Abstract

Abiotic stresses such as drought and nutrient availability can affect invertebrate herbivores feeding on plants, and potentially cascade up to impact their predators and parasitoids. Although these two factors separately been the subject of many studies, there are few tests of their combined effects in the context of pest species and their natural enemies on cultivated plants. Climate change models predict an increase in the frequency and severity of droughts, while the type and amount of fertiliser applied to crops is more under the control of growers. Understanding how these two abiotic factors may interact is key to utilising the potential of natural enemies to control pests under a future climate. To address this, a range of drought and fertiliser type treatments were applied to a model Brassica system in a factorial design, and the performance of two ubiquitous aphid species and their parasitoids was assessed. One aphid species was a specialist on Brassicas (*Brevicoryne brassicae*, with parasitoid *Diaeretiella rapae*) and the second a generalist aphid species (*Myzus persicae*, with parasitoid *Aphidius colemani*). The performance of both aphid species responded in a similar way to the treatments, and was maximised on plants growing in organic fertilisers under medium levels of drought stress. The strongest effects of drought and fertiliser cascaded up to affect parasitoids. Parasitoid performance responded in a broadly similar way to their aphid host performance in relation to fertiliser type. Some of the smaller effects of fertiliser treatments on aphid performance were not found for parasitoid performance. Aphid performance was greatest on plants under medium drought stress, but the parasitoids only responded consistently to the high drought stress treatment, on which their performance was reduced. Interactions between the drought and fertiliser did not have a large effect on aphid or parasitoid performance, compared with the strong main effects found for each treatment. These results are discussed in the context of previous and future research on the impacts of abiotic stresses on invertebrate herbivores and their natural enemies.

KEYWORDS
aphids, brassicas, climate change, parasitoids, plant-insect interactions

1 | INTRODUCTION

Abiotic stresses such as water availability (Tariq, Wright, Rossiter, & Staley, 2012), and the type and amount of nutrients supplied to a plant (Rowen, Tooker, & Blubaugh, 2019), have been shown to separately alter the performance and abundance of invertebrate herbivores feeding on plants, and can cascade up to affect their predators and parasitoids (Tariq, Wright, Bruce, & Staley, 2013a). In the context of pest species feeding on cultivated plants, the nutrients and water received by a host plant can affect populations of pests (Staley et al., 2010; Tariq et al., 2012) and the behaviour and performance of their natural enemies (Staley et al., 2011a; Tariq, Wright, et al., 2013a), thereby altering the amount of damage a plant receives and potentially also the crop yield.

Under current climate change predictions, rainfall is forecast to become increasingly variable in many regions of the globe, resulting in both more frequent periods of drought and more extreme precipitation events (Field et al., 2014). Several hypotheses have addressed the relationship between drought and plant–herbivore interactions. The plant stress hypothesis predicts that herbivore performance and populations increase under a range of "stress conditions," including drought, because of an increase in the availability of foliar nitrogen (White, 1969, 1984). This hypothesis was based largely on observational studies, in which phytophagous outbreaks were shown to coincide with dry, warm weather. Experimental tests of the plant stress hypothesis have produced varying results (Bultman & Faeth, 1987; Wheatley, Wightman, Williams, & Wheatley, 1989). Larson's modified plant stress hypothesis (the insect performance hypothesis) predicts that drought-stressed plants have a nonlinear relationship with herbivore performance, which is likely to be maximised under medium stress (Larsson, 1989). In the plant vigour hypothesis Price (1991) predicts that vigorous plants are more suitable hosts for herbivores than drought stressed plants because of the higher availability of nutrients (Cornelissen, Fernandes, & Vasconcellos-Neto, 2008; Price, 1991), lower abscission rates (Preszler & Price, 1995) or higher osmotic potential in vigorous shoots (Price, 2003).

Mopper and Whitham (1992) suggested that the duration of stress application may influence a herbivore's response to host-plant stress. Huberty and Denno (2004) built on this idea to put forward a "pulsed stress hypothesis," proposing that herbivores (particularly phloem feeders) benefit from plants that are periodically drought stressed, but perform less well on constantly stressed plants, because the loss of turgor pressure restricts the ability of sap feeders to access any increased nitrogen concentrations in the phloem. Tariq et al. (2012) tested the insect performance and pulsed stress hypotheses for two aphid species feeding on Brassica plants under drought stress treatments differing in severity and duration. Their results supported the insect performance hypothesis, but not the pulsed stress hypothesis (Tariq et al., 2012).

Fewer studies have assessed effects of drought on the performance or attack rates of parasitoids. Aslam, Johnson, and Karley (2013) found parasitism of the bird cherry–oat aphid, *Rhopalosiphum padi* L., was reduced on barley (*Hordeum vulgare* L.)

plants under drought treatments. Weldegergis, Zhu, Poelman, and Dicke (2015) found parasitoids on *Mamestra brassicae* caterpillars feeding on Brussels sprout (*Brassica oleracea* L. var gemmifera) were not affected by drought treatments.

As sustainable farming practices become more widespread (Adnan, Nordin, Bahruddin, & Tareq, 2019), interest is increasing in the effects of the type of fertiliser applied to crops on populations of herbivores and their natural enemies. A recent review of the effects of animal manures on arthropod pests found evidence that the use of animal manures, as opposed to conventional mineral fertilisers, can reduce the performance and abundance of pest species (Rowen et al., 2019). The use of animal manures was found to alter both bottom up processes, through changes to concentrations of both macro and micro-nutrients in plants, and to alter top down processes, because of changes in plant volatile signals to natural enemies and the soil surface habitat used by predators (Rowen et al., 2019).

An understanding of how drought may interact with other abiotic factors, such as the type or amount of fertiliser supplied to crops, would help to inform growers of the increased risks from pests which may occur under future climate change, and potentially to plan adaptation such as increased irrigation or use of more drought resistant varieties, in combination with different fertiliser types. However, to date the majority of studies testing the responses of pest species and their natural enemies to drought and the type of fertiliser supplied to their host plants have looked at each abiotic factor separately (Cividanes, Silva, Martins, & Cividanes, 2020; Stafford et al., 2012; Staley et al., 2011b; Tariq, Rossiter, Wright, & Staley, 2013b). Assessments of whether the two factors may interact to alter pest and natural enemy performance or behaviour are rare, although Griffith and Grinath (2018) tested the interacting effects of drought and conventional fertiliser on a tobacco system.

The family Brassicaceae contains several important crop species, and includes cabbages (*B. oleracea* L. var. capitata, Brassicaceae: Brassicales) which are grown in many countries (Hopkins, van Dam, & van Loon, 2009). *B. oleracea* var. capitata can be damaged by a wide variety of pests, including sap-sucking aphid species. *Brevicoryne brassicae* L. (Hemiptera: Aphididae, a specialist on Brassica species) and *Myzus persicae* Sulzer (Hemiptera: Aphididae, a generalist which feeds on a wide range of plant species) are two of the most ubiquitous aphid pests of *B. oleracea* var. capitata. Both of these aphid species have previously responded to drought stress and the type of fertiliser supplied to their host plants. For example, Cividanes et al. (2020) found populations of both aphid species were greater on kale grown in conventional fertiliser, compared with two organic fertilisers. The performance of both species increased on *B. oleracea* var. capitata plants under medium levels of drought stress, with reduced fecundity and intrinsic rates of increase on plants subjected to high levels of drought stress and unstressed controls (Tariq et al., 2012). In contrast, Khan, Ulrichs, and Mewis (2010) found *M. persicae* abundance was more affected by drought stress than the abundance of *B. brevicoryne* when feeding on kale, perhaps because of differences between the two crops or because only one level of drought was applied. Staley et al. (2010) found abundance of *B. brassicae* was increased on

B. oleracea var. capitata growing in organic chicken manure (CM) in a field trial, compared with those growing in conventional ammonium nitrate (AN) fertiliser, but that *M. persicae* abundance showed the opposite pattern. An experiment on potted *B. oleracea* growing under controlled conditions showed the performance of individual aphids of the two species responded in a similar way to the treatments as their abundance responded in the field trial (Stafford et al., 2012). These responses may be because of effects of fertiliser on the host plant chemistry, as *B. oleracea* var capitata fertilised with animal manure produced more glucosinolates, a group of chemicals which the specialist *B. brassicae* is well adapted to, than those plants growing in conventional AN fertiliser (Kazana et al., 2007; Staley et al., 2010).

Natural enemies of these two aphid species respond to both drought stress and the type of fertiliser (Banfield-Zanin, Rossiter, Wright, Leather, & Staley, 2012). Tariq, Wright, et al. (2013a) found that two parasitoid species (*Diaeretiella rapae* McIntosh, Hymenoptera: Braconidae and *Aphidius colemani* Viereck, Hymenoptera: Aphidiidae) parasitized a smaller percentage of potential aphid hosts, had lower percentage emergence, produced more males and had reduced longevity when they developed in aphids feeding on highly drought stressed plants. In contrast, Romo and Tylianakis (2013) found that parasitism of *B. brassicae* by *D. rapae* on kale (*B. oleracea* L. var. acephala) plants was positively affected by drought treatments. In relation to fertiliser type, Pope et al. (2012) found fewer *B. brassicae* were parasitized when feeding on *B. oleracea* var. capitata plants growing in animal manure, compared to those growing in conventional fertiliser. Thus, while the effects of drought stress and fertiliser type have both been well-studied in relation to aphids feeding on *B. oleracea* and their natural enemies, little is known about how the effects of these two factors may interact, and to our knowledge there has been no previous combined test on Brassicas and their pests.

Here, we test the combined effects of drought stress and fertiliser type on the performance of *B. brassicae* and *M. persicae*, and the performance of two parasitoid species (*D. rapae* and *A. colemani* respectively). Growers may apply more fertiliser to irrigated plants. However, in some areas including arid and semi-arid countries, water is at times in short supply (Feng et al., 2020). As droughts increase in both severity and frequency, the potential to compensation for drought with increased irrigation may be limited. In this context, understanding how pest aphid species and their parasitoids respond to fertiliser type under drought is critical. To achieve our objective, *B. oleracea* var. capitata plants were grown under three drought treatments (two levels of drought and an unstressed control), and in three types of fertiliser (including those used in conventional and organic horticulture) and an unfertilized control. The two treatments were applied in full factorial combination to plants growing in pots, and the performance of aphids and parasitoids assessed under controlled environment conditions.

Specifically, we tested the following hypotheses:

1. Performance of both aphid species will be increased when feeding on plants under medium drought stress compared to unstressed plants or those under high drought stress.

2. The two aphid species will respond differently to fertiliser type. *Brevicoryne brassicae* performance will be increased on plants grown in organic fertilisers, while the performance of *M. persicae* will be reduced.

3. The combination of medium drought stress and organic fertiliser type will result in the optimum performance of both *B. brassicae* aphids and their parasitoids *D. rapae*. In addition, *D. rapae* performance will be reduced under high drought stress.

4. In contrast, performance of the generalist aphid *M. persicae* and its parasitoid *A. colemani* will be maximised on plants growing under medium drought in conventional fertiliser. *Aphidius colemani* performance will be reduced under high drought stress.

2 | MATERIALS AND METHODS

2.1 | Study species

Brassica oleracea L. var. capitata cv. Saint (Sunny Seeds, Lahore, Pakistan) were sown in peat plugs and seedlings with first true leaves (BBCH growth stage 11; Meier, 2001) were transferred in pots (15.5 cm diameter) with unfertilized compost (peat, loam, sand and grit with 3:3:2:1 ratio) and were placed in a greenhouse (20 ± 5°C; 75% RH; LD 16:8 hr).

Myzus persicae and *B. brassicae* were collected from an infested field of *Brassica juncea* L. var. raya cv Anmol. Aphid cultures were maintained separately on *B. oleracea* stage 4 plants (BBCH-19; Andaloro, Rose, Shelton, Hoy, & Becker, 1983; Meier, 2001) grown in pots (15.5 cm diameter) with unfertilized compost containing peat, loam, sand and grit (3:3:2:1 ratio). Both aphid species were subcultured fortnightly and transferred to fresh plants. The parasitoid species *A. colemani* and *D. rapae* were collected from the same field as the aphids, and were reared on *M. persicae* and *B. brassicae* separately for at least two generations before being used in the main experiments, so that maternal host plant effects were avoided (Douloumpaka & van Emden, 2003). The insect cultures were maintained under controlled conditions (20 ± 2°C; 75% RH; LD 16:8 hr).

2.2 | Preliminary drought stress treatment trial

To assess the influence of different intensities of drought stress on leaf water content, four drought stress treatments were applied to *B. oleracea* plants, based on experience from previous studies on drought (Tariq et al., 2012). Seedlings with first true leaves (BBCH-11; Meier, 2001) were transferred to pots with unfertilized compost. Four weeks after transplanting, four water regimes (100, 200, 300 and 400 ml/week) were applied to stage 2 plants with five true leaves (BBCH-15). Plants were randomised in plastic trays on benches in a controlled environment room (20 ± 2°C; 75% RH; LD 16:8 hr). At stage 4 (9–12 true leaves; BBCH-19; 4 weeks of drought treatments), the plants were tested for leaf water content using the method in

Grace (1997). The results of this preliminary study were used to select drought stress treatments for the main experiment. All the plants were dead at 100 ml/week drought regime and thus this treatment was eliminated from the main experiment; plants were highly stressed at 200 ml/week, moderately stressed at 300 ml/week but no drought stress was observed at 400 ml/week water level.

2.3 | Experiment treatments

Treatments were allocated randomly to *B. oleracea* plants. Fertiliser treatments were applied first, through the addition of fertiliser to potting compost prior to transplanting *B. oleracea* seedlings into the plots. Plants were left to grow in the allocated fertiliser treatment before drought treatments were applied (details below).

Fertiliser treatments were selected as examples of frequently used types of organic and conventional fertilisers (Hanafi et al., 2018; Pope et al., 2012; Staley et al., 2010). Three types of fertiliser were applied by adding one of the following to 1 L of potting compost: (a) 6.67 g of CM with 4.5% nitrogen, (b) 5.88 g of spent mushroom compost (SMAS) with 7% nitrogen, (c) 0.86 g of AN with 34.5% nitrogen and (d) for the unfertilized control treatment nothing was added to the potting compost. After the fertiliser treatments were added to the compost, a *B. oleracea* seedling with first true leaves (BBCH-11) was transplanted into each pot. After transplanting, plants in pots were placed in plastic trays on benches in a controlled environment room (20 ± 2°C; 75% RH; LD 16:8 hr).

Three drought regimes were established on stage 2 plants with five true leaves (BBCH-15) as described above for the preliminary trial. The quantity of water added per pot per week was 400 ml for unstressed control plants, 300 ml for medium drought stress and 200 ml for high drought stress. Each drought treatment was applied in factorial combination with the four fertiliser treatments giving 12 factorial combinations of the two treatments. Each factorial combination was replicated five times for each aphid species. After 4 weeks of drought stress treatments, the plants at stage 4 (9–12 true leaves; BBCH-19) were used for aphid or parasitoid performance experiments.

2.4 | Aphid performance

Experimental drought and fertiliser treatments were randomly allocated in factorial combinations to plants at the same growth stages as described in Section 2.3, in plastic trays on benches in a controlled environment room (conditions as above). The performance of each aphid species was assessed using 120 *B. oleracea* plants: 10 replicates of each factorial treatment combination. The 120 plants were evenly split into two spatial blocks, with five plants per factorial treatment combination in each block. Aphid performance was measured as described in Stafford et al. (2012) and Tariq et al. (2012).

Three clip cages were fitted to the underside of first, second and third fully developed leaves on each plant to give 15 clip cages per treatment per aphid species per block. As the performance of alate and apterae can differ (Leather, 1989; Tariq, Wright, & Staley, 2010), the same form of aphid (apterae) was used throughout both experiments. After 16 to 18 hr (overnight), all aphids were removed leaving only one nymph per cage. For each aphid, the prereproductive time (*d*) and the number of offspring produced in the subsequent days (*Md*) were recorded and removed daily from each cage.

Data from both aphid species were recorded for the following parameters:

1. the effective fecundity, by allowing the aphids to reproduce during a period equivalent to the prereproductive time and
2. the intrinsic rate of increase $r_m = 0.738 \ (\ln Md)/d$ (Wyatt & White, 1977), where *Md* is the effective fecundity *d* is the prereproductive time.

2.5 | Parasitoid performance experiments

Experimental drought and fertiliser treatments were randomly allocated in factorial combinations to plants at the same growth stages as described in Section 2.3, in plastic trays on benches in a controlled environment room (conditions as above). The performance of each parasitoid species was assessed using 60 plants: five replicates of each factorial combination of drought and fertiliser treatments.

After 4 weeks of drought stress treatments, three clip cages were fitted to the underside of first, second and third fully developed leaves on each plant. Two separate batches of plants with five replicates per treatment were used for each aphid species. Clip cages and adult aphids were removed leaving one nymph per leaf on each treatment for 4 weeks. After 4 weeks (12-week-old plants) of aphid treatments, 300 aphids of each species were used to measure parasitoid performance. Extra aphids were removed from each plant. Plants infested with *B. brassicae* were used to assess the response of *D. rapae*; those infested with *M. persicae* were used with *A. colemani*.

Five paired parasitoids (one pair of female and male parasitoids per 60 aphids; Jarošík & Lapchin, 2001; Tariq, Wright, et al., 2013a) were released per replicate under ventilated bell cloches. After 24 hr, the parasitoids were removed and the remaining aphids were allowed to develop for 10 to 14 days to allow mummy formation (Tariq, Wright, et al., 2013a; van Emden & Kifle, 2002). Mummified aphids were collected in individual gelatine capsules and percent parasitism, percent emergence, sex ratio (proportion of males) and female hind tibiae length (Blande, Pickett, & Poppy, 2004; Tariq, Wright, et al., 2013a) were recorded.

2.6 | Statistical analyses

Within each aphid and parasitoid species, the effect of drought stress and fertiliser treatments and their interaction were analysed using two way factorial ANOVA. Prior to analysis, data for aphid performance (effective fecundity and intrinsic rate of increase) and

parasitoid performance (percentage emergence and female tibia length) were log root transformed. Least significant difference (LSD) tests were used to compare mean aphid and parasitoid performance between levels in a factor, to test the hypotheses above. These statistical analyses were carried out using SPSS statistical software version 16.

3 | RESULTS

## 3.1 | Preliminary drought stress trial: *B. oleracea* leaf relative water content

Leaf relative water content (mean ± *SEM*) per drought stress treatment was: 0.862 ± 0.018 for *B. oleracea* plants watered 400 ml/week; 0.767 ± 0.014 for plants watered 300 ml/week; 0.597 ± 0.016 for plants watered 200 ml/week and 0.492 ± 0.018 for those watered 100 ml/week. All plants watered with 100 ml water/week were dead or dying by the end of the trial, so this treatment was not used in the main experiments.

3.2 | Aphid performance

3.2.1 | Effective fecundity

There were significant differences in fecundity of *B. brassicae* for both drought stress ($F_{2,108}$ = 274.38, $p < .001$) and fertiliser treatments ($F_{3,108}$ = 53.72, $p < .001$; Figure 1). Fecundity of *B. brassicae* was greatest at medium drought stress, compared with both unstressed plants and those with high levels of drought stress (LSD, $p < .05$). Fecundity was intermediate on plants grown under high drought stress treatments, and was significantly different from unstressed plants (LSD, $p < .05$). The fecundity of *B. brassicae* was greater feeding on plants growing in each of the three fertilisers, compared with the unfertilised control plants (LSD, $p < .05$). *B. brassicae* fecundity was greatest on plants in CM and SMAS, and intermediate on plants in AN on which it differed significantly from both control and the two organic fertilisers (LSD, $p < .05$; Figure 1). The effective fecundity of *B. brassicae* was also significantly affected by a weak interaction between drought stress and fertiliser treatments ($F_{6,108}$ = 3.47, $p < .01$). This indicated that difference in overall fecundity because of drought stress depended on the particular fertiliser type. Overall, the fecundity of *B. brassicae* was greatest on plants with medium drought stress containing CM and SMAS fertilisers compared with the other drought and fertiliser treatments (LSD, $p < .05$; Figure 1).

There were significant differences in fecundity of *M. persicae* dependent upon both drought stress ($F_{2,108}$ = 317.29, $p < .001$) and fertiliser treatments ($F_{3,108}$ = 105.39, $p < .001$). Fecundity of *M. persicae* was significantly greater at medium drought stress compared with unstressed plants. Fecundity was intermediate for those on plants under the high drought stress treatment, and differed significantly from both the other two treatments (LSD, $p < .05$). The
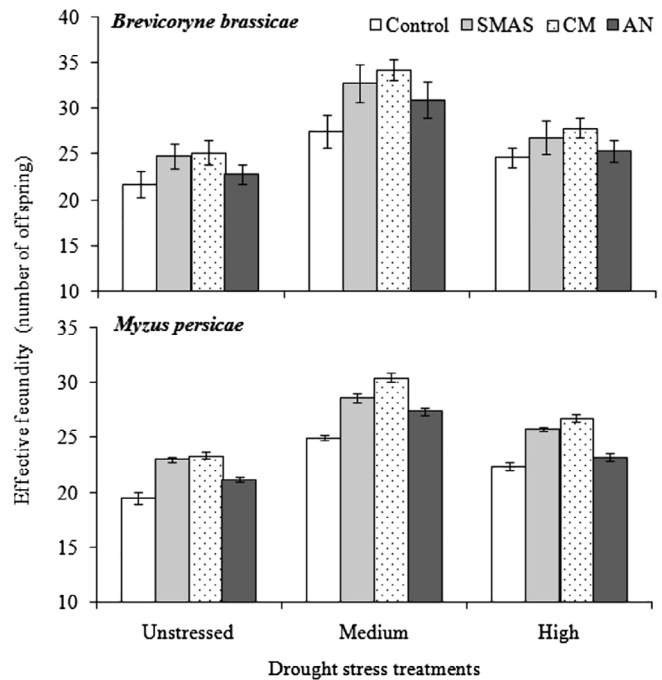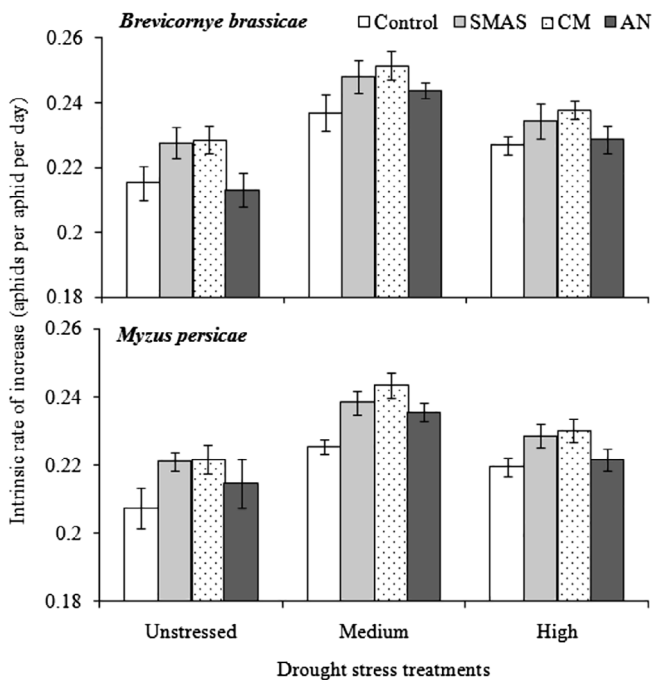

FIGURE 1 Effective fecundity of *Brevicoryne brassicae* and *Myzus persicae* (mean ± LSD) feeding on *Brassica oleracea* L. var. capitata cv. Saint plants under different levels of drought stress (unstressed, medium and high) and fertiliser treatments (AN, ammonium nitrate; C, control without fertiliser; CM, chicken manure; SMAS, spent mushroom compost)

fecundity of *M. persicae* was greater on all three fertiliser treatments, compared to the unfertilized control plants, and also differed significantly between each of the three fertiliser treatments. *M. persicae* fecundity was greatest on plants growing in CM fertiliser, followed by SMAS, then AN and lowest on unfertilized control plants (LSD, $p < .05$; Figure 1). There was no significant interaction between the effects of drought stress and fertiliser on the fecundity of *M. persicae*.

3.2.2 | Intrinsic rate of increase

Intrinsic rate of increase for both aphid species followed a similar pattern to aphid fecundity. There were significant differences in intrinsic rate of increase for *B. brassicae* that depended upon both drought stress ($F_{2,108}$ = 294.94, $p < .001$) and fertiliser treatments ($F_{3,108}$ = 59.37, $p < .001$). Unlike for fecundity, the intrinsic rate of increase for *B. brevicoryne* feeding on plants growing in AN did not differ significantly from unfertilized control plants (Figure 2). The rate of increase for *B. brassicae* was also significantly affected by an interaction between drought stress and fertiliser treatments, but the interaction effect was small compared to the main effects ($F_{6,108}$ = 3.47, $p < .01$). Similar to *B. brevicoryne*, there were significant differences in intrinsic rate of increase for *M. persicae* of both the drought stress ($F_{2,108}$ = 308.99, $p < .001$) and fertiliser treatments ($F_{3,108}$ = 98.34, $p < .001$).

Intrinsic rate of increase for both aphid species was significantly greatest at medium drought stress, compared with plants in the

FIGURE 2 Intrinsic rate of increase of *Brevicoryne brassicae* and *Myzus persicae* (mean ± LSD) feeding on *Brassica oleracea* L. var. capitata cv. Saint plants under different levels of drought stress (unstressed, medium and high) and fertiliser treatments (AN, ammonium nitrate; C, control without fertiliser; CM, chicken manure; SMAS, spent mushroom compost)

unstressed treatment. Intrinsic rate of increase was intermediate on plants under high drought stress treatments, and significantly different from the medium drought stress and unstressed treatments (LSD, $p < .05$). Intrinsic rate of increase of *M. persicae* differed significantly between all four fertiliser treatments, and was greatest on plants growing in CM, followed by SMAS, AN and lowest on unfertilized control plants (LSD, $p < .05$). Intrinsic rate of increase for *M. persicae* was significantly affected by the interaction between drought stress and fertiliser treatments ($F_{6,108} = 3.21$, $p < .01$); on control plants in the unstressed treatment, there was no significant difference in intrinsic rate of increase of *M. persicae* on plants growing in CM or SMAS.

3.3 | Parasitoid performance

3.3.1 | Percentage parasitism

Percentage parasitism of *B. brassicae* by *D. rapae* was significantly different depending upon the drought stress ($F_{2,48} = 65.21$, $p < .001$) and fertiliser treatment ($F_{3,48} = 17.23$, $p < .001$). The percentage parasitism by *D. rapae* differed significantly between each drought stress treatment (LSD, $p < .05$), and was greatest at medium drought stress, followed by unstressed plants and lowest under high drought stress treatments. Percentage parasitism by *D. rapae* was greatest on aphids feeding on plants growing in either CM or SMAS, compared with both control plants and those fertilised with AN (LSD, $p < .05$; Figure 3a).

Percentage parasitism of *M. persicae* by *A. colemani* differed significantly with both the drought stress ($F_{2,48} = 92.27$, $p < .001$) and fertiliser treatments ($F_{3,48} = 27.30$, $p < .001$). The percentage parasitism by *A. colemani* was significantly lower at high drought stress compared with both medium drought stress and unstressed plants (LSD, $p < .05$), but did not differ between plants under medium drought stress and unstressed plants (Figure 4a). Percentage parasitism was highest on plants growing in CM or SMAS, compared with unfertilised control plants or those growing in AN (LSD, $p < .05$; Figure 4a).

3.3.2 | Percentage emergence

Percentage emergence of *D. rapae* was significantly affected by drought stress ($F_{2,48} = 74.58$, $p < .001$) and fertiliser treatment ($F_{3,48} = 29.75$, $p < .001$). Percentage emergence of *D. rapae* was significantly greater at both medium drought stress and unstressed treatments compared with high drought stress (LSD, $p < .05$), but there was no significant difference in parasitoid emergence between medium drought stress and unstressed plants. Percentage emergence of *D. rapae* was maximised from aphids on plants growing in CM and SMAS, and significantly lower from aphids on plants that were unfertilised controls or growing in AN treatments (LSD, $p < .05$; Figure 3b).

Percentage emergence of *A. colemani* was dependent upon the drought stress ($F_{2,48} = 27.74$, $p < .001$) and fertiliser treatments ($F_{3,48} = 16.25$, $p < .001$). Percentage emergence of *A. colemani* was significantly higher on medium drought stress and unstressed plants compared with high drought stress (LSD, $p < .05$; Figure 4b). Percentage parasitism differed significantly between each of the four fertiliser treatments, and was highest on plants growing in CM, followed by SMAS, AN and lowest on the unfertilised control plants. There was no interaction between the effects of drought stress and fertiliser treatments on percentage emergence for either parasitoid species.

3.3.3 | Sex ratio

The main effects of drought stress ($F_{2,48} = 88.82$, $p < .001$) and fertiliser types ($F_{3,48} = 11.87$, $p < .001$) were significant for the sex ratio of *D. rapae*. The proportion of males was significantly greater at high drought stress compared with unstressed plants and high drought stress (LSD, $p < .05$; Figure 3c). In relation to fertiliser, the lowest male proportion of *D. rapae* was observed on plants growing in CM and SMAS, compared with control and AN (LSD, $p < .05$). The main effects of drought stress ($F_{2,48} = 231.65$, $p < .001$) and fertiliser types ($F_{3,48} = 16.01$, $p < .001$) also had a significant effect on the sex ratio of *A. colemani*. The proportion of males was significantly greater at high drought stress compared with unstressed plants and medium drought stress (LSD, $p < .05$; Figure 4c). The lowest male proportion of *A. colemani* was observed on plants in CM, followed by SMAS, compared with both AN and unfertilised control plants (LSD, $p < .05$). There was no interaction between the effects of drought stress and

FIGURE 3 Performance of the parasitoid *Diaeretiella rapae* (mean ± LSD) of *Brevicoryne brassicae* reared on *Brassica oleracea* L. var. capitata cv. Saint plants under different levels of drought stress (unstressed, medium and high) and fertiliser treatments (AN, ammonium nitrate; C, control without fertiliser; CM, chicken manure; SMAS, spent mushroom compost). (a) Percentage parasitism, (b) percentage emergence, (c) sex ratio and (d) female tibia length (mm). A high sex ratio indicates a high proportion of male parasitoids
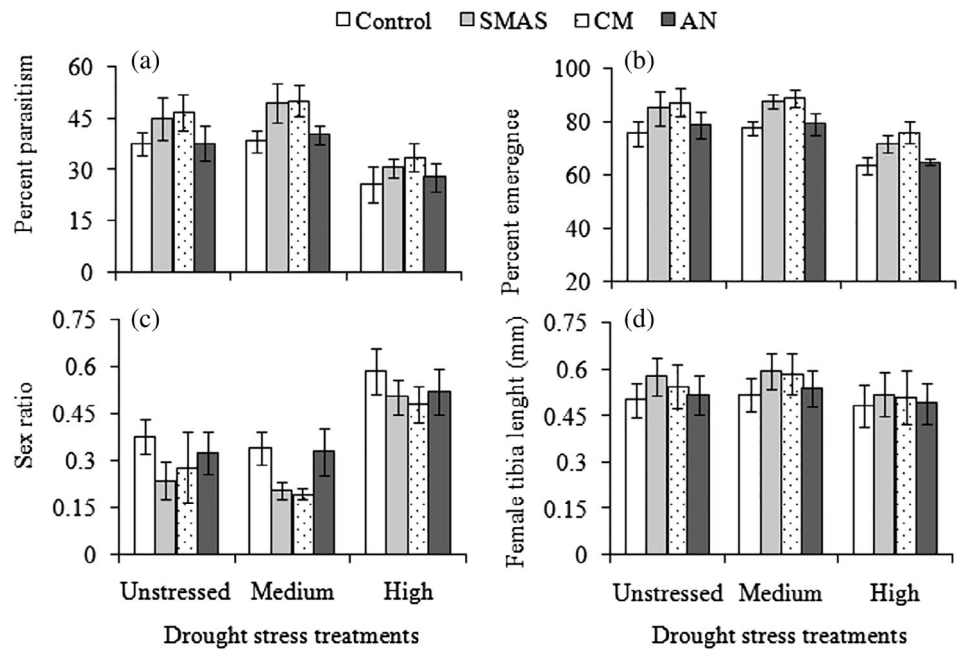
FIGURE 4 Performance of parasitoid *Aphidius colemani* (mean ± LSD) of *Myzus persicae* reared on *Brassica oleracea* L. var. capitata cv. Saint plants under different levels of drought stress (unstressed, medium and high) and fertiliser treatments (AN, ammonium nitrate; C, control without fertiliser; CM, chicken manure; SMAS, spent mushroom compost). (a) Percentage parasitism, (b) percentage emergence, (c) sex ratio and (d) female tibia length (mm). A high sex ratio indicates a high proportion of male parasitoids
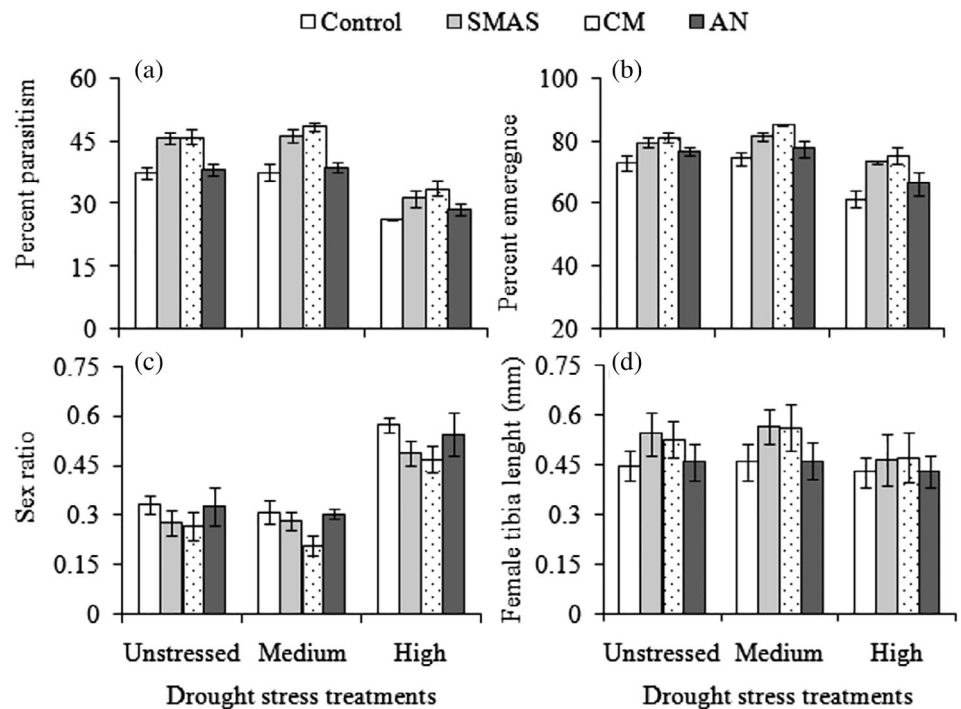
fertiliser treatments on percentage emergence for either parasitoid species.

3.3.4 | Female tibia length

The impacts of drought stress ($F_{2,348}$ = 23.20, $p < .001$) and fertiliser treatment ($F_{3,348}$ = 8.70, $p < .001$) were also significant for the tibia length of female *D. rapae*. Female tibia length of *D. rapae* was longest at medium drought stress compared with the other two drought treatments, intermediate on unstressed plants and shortest on plants under high drought stress (all three levels of drought treatment differed significantly, LSD, $p < .05$; Figure 3d). Female tibia length was longest on the CM and SMAS treatments, which differed from the control and AN (LSD, $p < .05$; Figure 3d). The interaction between drought stress and fertiliser treatment ($F_{6,348}$ = 5.38, $p < .001$) on female tibia length of *D. rapae* was significant.

Both drought stress ($F_{2,348}$ = 35.75, $p < .001$) and fertiliser treatments ($F_{3,348}$ = 50.02, $p < .001$) had significant effects on the tibia length of female *A. colemani*. Female tibia length was longest at

medium drought stress, intermediate on unstressed plants and shortest under the high drought stress treatments (LSD, $p < .05$; Figure 4d). Female tibia length was also greatest on CM and SMAS, which differed significantly from shorter tibia lengths under both control and AN treatments (LSD, $p < .05$; Figure 4d). Female tibia length of *A. colemani* was significantly affected by an interaction between drought stress and fertiliser treatment ($F_{6,348} = 3.24$, $p < .001$).

4 | DISCUSSION

The effects of drought stress and fertiliser were broadly similar for the two aphid species. Performance of individual aphids of both species, in terms of their fecundity and intrinsic rate of increase, was greatest at medium drought stress, intermediate at high drought stress and lowest on plants that were not subjected to drought stress, as found previously by Tariq et al. (2012). These results support our first hypothesis above, that performance of both aphid species will be increased when feeding on plants under medium drought stress. More broadly, our results also provide support for Larsson's (1989) insect performance hypothesis.

In relation to the type of fertiliser supplied to their host plant, performance of both aphid species was overall greatest on the two organic fertilisers (CM and SMAS), lowest on the unfertilized control plants and intermediate on plants fertilised with conventional AN fertiliser. There were minor variations in this general pattern, in that for *B. brassicae* the intrinsic rate of increase did not differ significantly between unfertilized host plants and those growing in AN. For *M. persicae*, the intrinsic rate of increase also differed significantly between the two types of organic fertiliser (greatest on CM). This similar response shown by the two aphid species to the type of fertiliser is different to previous studies, in which *M. persicae* abundance (Staley et al., 2010) and performance (Stafford et al., 2012) were both greater on plants growing in AN than those in CM, but *B. brassicae* showed the reverse pattern. This difference may be because of varying composition of different sources of organic fertiliser, or variation in the response of *B. brassicae* populations from different countries to plant glucosinolate and nitrogen content. Given this previous work, the broadly similar response of the aphid species to fertiliser type was unexpected, and leads to the rejection of our second hypothesis which predicted the two aphid species would respond differently to fertiliser type.

Performance of the two parasitoid species also showed broadly similar responses to both the drought stress and fertiliser treatments. Parasitoid performance in relation to drought treatments was reduced most under high drought, for all four variables tested. Some performance variables (% parasitism of *D. rapae*; sex ratio of *A. colemani* and female tibia length for both species) were greatest under medium drought stress, while the majority of parasitoid performance variables did not differ significantly between medium drought stress and unstressed control plants. While the effects of all three levels of drought treatment significantly affected aphid performance, only the high drought stress treatment cascaded up to consistently reduce

parasitoid performance. Tariq, Wright, et al. (2013a) also found performance of these two parasitoid species was reduced under high drought stress compared with unstressed control plants, in line with the results here, but did not assess the effects of medium drought stress. Tariq et al. (2012) found glucosinolate concentrations were increased under drought stress and greatest under high drought stress, using the same plant and aphid species as the current study. *B. brassicae* can sequester glucosinolates from their host plants and use them in defence against natural enemies (Pope et al., 2012), so it is possible *D. rapae* performance was reduced on high drought stress plants because of increased availability of glucosinolates for sequestration. However, *M. persicae* is not thought to sequester glucosinolates, so this cannot be the mechanism by which the performance of *A. colemani* is reduced under high drought stress.

Performance of both parasitoid species was better on plants growing in the two organic fertiliser types, compared with both the unfertilized plants and those growing in conventional (AN) fertiliser. There were some minor additional results to this broad pattern, in that % emergence of *A. colemani* also differed significantly across all the four fertiliser types, and sex ratio was slightly greater on plants in CM than SMAS. As for the drought treatments, the largest effects of fertiliser type found to affect aphid performance (organic fertiliser vs. conventional or unfertilized) also altered the performance of their parasitoids, in a similar way for the two parasitoid species. However, the smaller fertiliser effects on aphid performance (between AN fertiliser and unfertilized controls) were not consistently found for performance of the two parasitoid species. This shows that while large effects of abiotic treatments on herbivore performance may alter their quality to affect parasitoid performance, not all the smaller effects cascade up to the next trophic level. Pope et al. (2012) found a lower rate of parasitism by *D. rapae* on *B. brevicoryne* feeding on Brassica plants grown in CM, when compared with those growing in a conventional John Innes fertiliser. This appears broadly in contrast with results in the current study, perhaps because of differences in fertiliser composition, although the two studies are not directly comparable as the current study did not include John Innes fertiliser.

The third hypothesis above predicted that performance of *B. brevicoryne* and its parasitoid *D. rapae* would be maximised on plants growing in organic fertiliser under medium drought stress, and that is supported by our results. However, as discussed in relation to aphid performance, for *M. persicae* we predicted performance would be greater on plants in conventional than organic fertiliser, and that this pattern would be similar for its parasitoid *A. colemani* (hypothesis 4). Our results show the two aphid species and two parasitoid species have broadly similar responses to both the fertiliser type and drought stress treatments, so the fourth hypothesis is rejected.

Significant interactions between fertiliser and drought stress treatments were found for most of the aphid performance variables, but the interaction effects were much smaller than the main effects, with interaction F values typically between 10 and 80 times smaller than those of the main treatments. Where interactions were found, generally they showed that differences between fertiliser type were not apparent for one of the three levels of drought stress treatment,

rather than indicating opposing effects. Fewer interaction effects were found for parasitoid performance than for performance of their aphid hosts. Broadly, interactions between these two types of abiotic stress do not appear to be as important for the performance of these aphid species and their parasitoids as the separate effects of drought and fertiliser type.

To our knowledge, no previous study has addressed the effects of drought stress and fertiliser type on aphids and their parasitoids in a Brassica system. Griffith and Grinath (2018) assessed the effects of drought and fertiliser amount on an aphid and a caterpillar species on tobacco plants. Both a caterpillar and an aphid species were found to be more responsive to drought than to the amount of fertiliser, although results differed slightly with the tobacco variety (Griffith & Grinath, 2018). Further studies on the interaction between water and nutrient availability on pest species and their natural enemies are needed across a range of crop species, and in field trial conditions, to understand if the patterns found here may be more broadly applicable under a future climate.

REFERENCES

Andaloro, J. T., Rose, K. B., Shelton, A. M., Hoy, C. W., & Becker, R. F. (1983). Cabbage growth stages. *New York's Food and Life Sciences Bulletin*, *101*, 1–4.

Adnan, N., Nordin, S. M., Bahruddin, M. A., & Tareq, A. H. (2019). A state-of-the-art review on facilitating sustainable agriculture through green fertilizer technology adoption: Assessing farmers behavior. *Trends in Food Science & Technology*, *86*, 439–452.

Aslam, T. J., Johnson, S. N., & Karley, A. J. (2013). Plant-mediated effects of drought on aphid population structure and parasitoid attack. *Journal of Applied Entomology*, *137*, 136–145.

Banfield-Zanin, J. A., Rossiter, J. T., Wright, D. J., Leather, S. R., & Staley, J. T. (2012). Predator mortality depends on whether its prey feeds on organic or conventionally fertilised plants. *Biological Control*, *63*, 56–61.

Blande, J. D., Pickett, J. A., & Poppy, G. M. (2004). Attack rate and success of the parasitoid *Diaeretiella rapae* on specialist and generalist feeding aphids. *Journal of Chemical Ecology*, *30*, 1781–1795.

Bultman, T. L., & Faeth, S. H. (1987). Impact of irrigation and experimental drought stress on leaf-mining insects of Emory oak. *Oikos*, *48*, 5–10.

Cividanes, F. J., Silva, K. P.d., Martins, I. C. F., & Cividanes, T. M.d. S. (2020). Phytophagous insects and natural enemies in kale under organic and chemical fertilizers. *Arquivos do Instituto Biológico*, *87*, e0902018.

Cornelissen, T., Fernandes, G. W., & Vasconcellos-Neto, J. (2008). Size does matter: Variation in herbivory between and within plants and the plant vigor hypothesis. *Oikos*, *117*, 1121–1130.

Douloumpaka, S., & van Emden, H. F. (2003). A maternal influence on the conditioning to plant cues of *Aphidius colemani* Viereck, parasitizing the aphid *Myzus persicae* Sulzer. *Physiological Entomology*, *28*, 108–113.

Feng, J., Hussain, H. A., Hussain, S., Shi, C., Cholidah, L., Men, S., ... Wang, L. (2020). Optimum water and fertilizer management for better growth and resource use efficiency of rapeseed in rainy and drought seasons. *Sustainability*, *12*, 703.

Field, C. B., Barros, V. R., Mastrandrea, M. D., Mach, K. J., Abdrabo, M. A.-K., Adger, W. N., ... Yohe, G. W. (2014). IPCC, 2014: Summary for policymakers. In C. B. Field, V. R. Barros, D. J. Dokken, K. J. Mach, M. D. Mastrandrea, T. E. Bilir, et al. (Eds.), *Climate change 2014: Impacts, adaptation, and vulnerability. Part A: Global and sectoral aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 1–32). Cambridge, UK: Cambridge University Press.

Grace, J. (1997). Plant water relations. In M. J. Crawley (Ed.), *Plant ecology* (2nd ed., pp. 28–50). Oxford, UK: Blackwell Science.

Griffith, K. A., & Grinath, J. B. (2018). Interactive effects of precipitation and nitrogen enrichment on multi-trophic dynamics in plant-arthropod communities. *Plos One*, *13*, e0201219.

Hanafi, F. H. M., Rezania, S., Taib, S. M., Din, M. F. M., Yamauchi, M., Sakamoto, M., ... Ebrahimi, S. S. (2018). Environmentally sustainable applications of agro-based spent mushroom substrate (SMS): An overview. *Journal of Material Cycles and Waste Management*, *20*, 1383–1396.

Hopkins, R. J., van Dam, N. M., & van Loon, J. J. A. (2009). Role of glucosinolates in insect-plant relationships and multitrophic interactions. *Annual Review of Entomology*, *54*, 57–83.

Huberty, A. F., & Denno, R. F. (2004). Plant water stress and its consequences for herbivorous insects: A new synthesis. *Ecology*, *85*, 1383–1398.

Jarošík, V., & Lapchin, L. (2001). An experimental investigation of patterns of parasitism at three spatial scales in an aphid-parasitoid system (Hymenoptera: Aphidiidae). *European Journal of Entomology*, *98*, 295–299.

Kazana, E., Pope, T. W., Tibbles, L., Bridges, M., Pickett, J. A., Bones, A. M., ... Rossiter, J. T. (2007). The cabbage aphid: A walking mustard oil bomb. *Proceedings of the Royal Society B-Biological Sciences*, *274*, 2271–2277.

Khan, M. A. M., Ulrichs, C., & Mewis, I. (2010). Influence of water stress on the glucosinolate profile of *Brassica oleracea* var. *italica* and the performance of *Brevicoryne brassicae* and *Myzus persicae*. *Entomologia Experimentalis et Applicata*, *137*, 229–236.

Larsson, S. (1989). Stressful times for the plant stress – Insect performance hypothesis. *Oikos*, *56*, 277–283.

Leather, S. R. (1989). Do alate aphids produce fitter offspring? The influence of maternal rearing history and morph on life-history parameters of *Rhopalosiphum padi* (L.). *Functional Ecology*, *3*, 237–244.

Meier, U., (2001). Growth stages of mono and dicotyledonous plants. BBCH Monograph. 2nd ed. *German Federal Biological Research Centre for Agriculture and Forestry*. Braunschweig, Germany, pp. 158.

Mopper, S., & Whitham, T. G. (1992). The plant stress paradox – Effects on pinyon sawfly sex ratios and fecundity. *Ecology*, *73*, 515–525.

Pope, T. W., Girling, R. D., Staley, J. T., Trigodet, B., Wright, D. J., Leather, S. R., ... Poppy, G. M. (2012). Effects of organic and conventional fertilizer treatments on host selection by the aphid parasitoid *Diaeretiella rapae*. *Journal of Applied Entomology*, *136*, 445–455.

Preszler, R. W., & Price, P. W. (1995). A test of plant-vigour, plant-stress, and plant-genotype effects on leaf-miner oviposition and performance. *Oikos*, *74*, 485–492.

Price, P. W. (1991). The plant vigour hypothesis and herbivore attack. *Oikos*, *62*, 244–251.

Price, P. W. (2003). *Macroevolutionary theory on macroecological patterns*. Cambridge, UK: Cambridge University Press.

Romo, C. M., & Tylianakis, J. M. (2013). Elevated temperature and drought interact to reduce parasitoid effectiveness in suppressing hosts. *PLoS One*, *8*, e58136.

Rowen, E., Tooker, J. F., & Blubaugh, C. K. (2019). Managing fertility with animal waste to promote arthropod pest suppression. *Biological Control*, *134*, 130–140.

Stafford, D. B., Tariq, M., Wright, D. J., Rossiter, J. T., Kazana, E., Leather, S. R., ... Staley, J. T. (2012). Opposing effects of organic and conventional fertilizers on the performance of a generalist and a specialist aphid species. *Agricultural and Forest Entomology*, *14*, 270–275.

Staley, J. T., Girling, R. D., Stewart-Jones, A., Poppy, G. M., Leather, S. R., & Wright, D. J. (2011a). Organic and conventional fertilizer effects on a tritrophic interaction: Parasitism, performance and preference of *Cotesia vestalis*. *Journal of Applied Entomology*, *135*, 658–665.

Staley, J. T., Stafford, D. B., Green, E. R., Leather, S. R., Rossiter, J. T., Poppy, G. M., & Wright, D. J. (2011b). Plant nutrient supply determines competition between phytophagous insects. *Proceedings of the Royal Society B-Biological Sciences*, 278, 718–724.

Staley, J. T., Stewart-Jones, A., Pope, T. W., Wright, D. J., Leather, S. R., Hadley, P., ... Poppy, G. M. (2010). Varying responses of insect herbivores to altered plant chemistry under organic and conventional treatments. *Proceedings of the Royal Society B-Biological Sciences*, 277, 779–786.

Tariq, M., Wright, D. J., Bruce, T. J. A., & Staley, J. T. (2013a). Drought and root herbivory interact to alter the response of above-ground parasitoids to aphid infested plants and associated plant volatile signals. *PLoS One*, 8, e69013.

Tariq, M., Rossiter, J. T., Wright, D. J., & Staley, J. T. (2013b). Drought alters interactions between root and foliar herbivores. *Oecologia*, 172, 1095–1104.

Tariq, M., Wright, D. J., Rossiter, J. T., & Staley, J. T. (2012). Aphids in a changing world: Testing the plant stress, plant vigour and pulsed stress hypotheses. *Agricultural and Forest Entomology*, 14, 177–185.

Tariq, M., Wright, D. J., & Staley, J. T. (2010). Maternal host plant effects on aphid performance: Contrasts between a generalist and a specialist species on Brussels sprout cultivars. *Agricultural and Forest Entomology*, 12, 107–112.

van Emden, H. F., & Kifle, A. T. (2002). Performance of the parasitoid *Aphidius colemani* when reared on *Myzus persicae* on a fully defined artifical diet. *BioControl*, 47, 607–616.

Weldegergis, B. T., Zhu, F., Poelman, E. H., & Dicke, M. (2015). Drought stress affects plant metabolites and herbivore preference but not host location by its parasitoids. *Oecologia*, 177, 701–713.

Wheatley, A. R. D., Wightman, J. A., Williams, J. H., & Wheatley, S. J. (1989). The influence of drought stress on the distribution of insects on four groundnut genotypes grown near Hyderabad, India. *Bulletin of Entomological Research*, 79, 567–577.

White, T. C. R. (1969). An index to measure weather-induced stress of trees associated with psyllids in Australia. *Ecology*, 50, 905–909.

White, T. C. R. (1984). The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia*, 63, 90–105.

Wyatt, I. J., & White, P. F. (1977). Simple estimation of intrinsic increase rates for aphids and tetranychid mites. *Journal of Applied Ecology*, 14, 757–766.