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RESEARCH ARTICLE

Silicon supplementation and jasmonate activation synergistically increase phenolic defences against a legume herbivore

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Abstract

1. The accumulation of silicon (Si) is widely reported to have anti-herbivore defensive properties in grasses. There is emerging, but fragmentary, evidence that Si could play a similar role in legumes.
2. Here, we sought to understand the effects of Si supplementation on anti-herbivore defensive properties in lucerne (*Medicago sativa*), especially in relation to other potential defences (i.e. phenolics) and the phytohormone that regulates anti-herbivore defences, jasmonic acid or jasmonate (JA), which is also linked to Si accumulation.
3. We determined how growth, root nodulation and chemistry (carbon, nitrogen and phenolic concentrations) of four genotypes of lucerne responded to Si supplementation, with and without the application of JA, and we used feeding assays to determine the subsequent effects on the feeding success of adult *Sitona discoideus* weevils.
4. Si supplementation increased plant mass and root nodulation of *M. sativa* by 61% and 227%, respectively, and reduced relative consumption (RC) and frass production by *S. discoideus* by 38% and 30% respectively. Si supplementation had no effect on foliar nitrogen concentrations, most likely due to the dilution effects of increased plant growth and foliar carbon. Phenolic concentrations were negatively correlated with leaf RC; RC also decreased by 34% when JA was applied to plants. When Si was combined with JA application, phenolics were significantly enhanced, demonstrating the potential to stimulate multiple anti-herbivore properties in *M. sativa*. Overall, the Si- and JA-induced phytochemical and herbivore feeding responses were consistent between the four lucerne genotypes tested.
5. *Synthesis*. The novel findings suggest that Si accumulation may play a more important role in legume resistance to herbivorous animals than previously thought. The ubiquity of soil Si and its emerging functional role in plant biology, including

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plant–animal interactions, suggest that these patterns could be common among legumes.

KEYWORDS
herbivory, insects, jasmonic acid, phenolics, plant defence, silicon

1 | INTRODUCTION

Silicon (Si) accumulation by plants is now recognised as a key mechanism for alleviating the adverse effects of environmental stresses, including drought, metal toxicity, disease and herbivory (Cooke & Leishman, 2016; Debona et al., 2017). Si is taken up by roots as monosilicic acid, $Si(OH)_4$, and subsequently translocated and deposited in various plant tissues, within and between cells, in the form of hydrated biogenic silica, $SiO_2{\cdot}nH_2O$ (Raven, 1983). Si defences against herbivores include abrasive effects on their mouthparts and diminished nutrient acquisition via reduced palatability and digestibility of foliage (Hartley & DeGabriel, 2016; Massey & Hartley, 2006).

The vast majority of research into the beneficial effects of Si supplementation of plants is concerned with the grasses (Poaceae) due to many of them being hyper-accumulators of Si (Hodson et al., 2005; Johnson, Hartley, et al., 2021; Massey et al., 2006). A recent review, however, revealed that Si supplementation of legumes (Fabaceae) alleviated similar environmental stresses as grasses (Putra et al., 2020). Legumes are the most valuable source of protein for crops and animal feed, and also provide important habitats for beneficial organisms (e.g. pollinators; Stagnari et al., 2017). They possess root nodules that house nitrogen (N)-fixing rhizobial bacteria, which increase nitrogen availability for legumes and subsequent or adjacent non-N-fixing plants (Hirsch et al., 2001; Ta & Faris, 1987). Legume herbivores, such as *Sitona* weevils, chew plant tissue below- and above-ground. *Sitona* larvae feed on the root nodules and have the capacity to alter interactions between plants via nitrogen transfer (Murray & Hatch, 1994).

A key knowledge gap that Putra et al. (2020) identified was how little is known about whether Si alleviated the impacts of herbivory in legumes. To our knowledge, only one study has investigated the impacts of Si supplementation of a legume (soybean; *Glycine max*) on an insect herbivore chewer, the feeding guild most affected by Si defences (Johnson, Hartley, et al., 2021), which reported reduced chewing herbivore growth rates (Johnson et al., 2020). Other feeding guilds, such as cell- and sap-feeding arthropods have similarly been negatively impacted by Si supplementation of soybean (Ferreira & Moraes, 2011) and the common bean (*Phaseolus vulgaris*; Peixoto et al., 2011).

In addition to providing physical defence against herbivores, Si supplementation is known to alter the jasmonate (jasmonic acid [JA]) pathway (Hall et al., 2019), which is the master regulator of secondary metabolite defences (e.g. phenolics, a broad family of compounds, many of which play a role in anti-herbivore defence) against chewing arthropods (Puentes et al., 2021). Stimulation of the JA pathway also causes rapid induction of Si defences, which can act synergistically with other plant compounds to affect herbivore ingestion and digestion of plant tissue (Johnson, Hartley, et al., 2021; Waterman et al., 2021; Ye et al., 2013). As such, understanding the combined effects of Si and JA on herbivore feeding and the associated plant chemical responses is important for predicting how plants will respond to herbivore pest attack and how we can use this to reduce damage to plants. Studies (Cooke & Leishman, 2012 and references therein) have demonstrated that Si-accumulating grasses have lower concentrations of phenolic compounds, suggesting that Si can trade-off with phenolic concentrations. This trade-off may occur because they have similar roles in plants and/or because Si is used as a metabolically cheaper defence against herbivores (Cooke & Leishman, 2011; Schaller et al., 2012). However, the capability of Si to alter the JA pathway and the production of chemical defences (e.g. anti-herbivore phenolic compounds) is limited and is likely to be an indirect response (i.e. Si does not react with chemical pathways directly), for which the underlying mechanisms remain unclear (Hall et al., 2019). Legumes can contain high concentrations of phenolic compounds, especially tannins (Barbehenn & Constabel, 2011; Horvat et al., 2022), but accumulate relatively small concentrations of Si compared with the grasses (Putra et al., 2020), so it is possible that the trade-off between C-based defences and Si is less apparent if present at all. Moreover, activation of the JA pathway decoupled the negative relationship between Si and phenolic defences in the grass *Brachypodium distachyon* (Waterman et al., 2021) so the relationship between Si accumulation and phenolics is somewhat plastic.

Si supplementation frequently increases nodule abundance, nitrogenase activity (indicative of increased N fixation) and concentrations of nitrogenous compounds (protein and amino acids) in *Medicago* spp. and other legumes (Izaguirre-Mayoral et al., 2017; Nelwamondo & Dakora, 1999; Putra et al., 2022), especially when plants are under stress (e.g. Al Murad & Muneer, 2022). The mechanisms of these responses remain unknown but may be associated with an increased provision of carbon-derived compounds from the legume to the rhizobial bacteria due to the substitution of structural carbon with Si and/or an increased transport of solute and gases from soil due to increased nodule permeability (Putra et al., 2020). Moreover, Si-induced increases in nodulation may depend on the capability of some legumes to take up Si or the level of mutualism in the legume–rhizobia symbiosis (Putra et al., 2022). A Si-induced increase in plant N could potentially undermine the effectiveness of Si in increasing legume resistance to herbivory because N is usually the limiting nutrient in insect herbivore diets (Mattson, 1980); increased N availability via Si supplementation could therefore increase susceptibility to herbivory.

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It could be envisaged that Si supplementation of legumes could affect herbivorous insects in a multitude of ways, which we conceptually depict in Figure 1. In summary, Si supply may promote leaf silicification and Si-based defences in the leaves (mechanism 1; Figure 1) and could either increase or decrease concentrations of secondary metabolite defences such as phenolics (mechanism 2; Figure 1). Concurrently, stimulation of the JA pathway may promote Si accumulation and phenolic defences (mechanism 3; Figure 1), both of which may negatively affect herbivore feeding and performance. An added consideration for legumes, compared with non-leguminous plants, is that Si supplementation may also make plants more nutritious to herbivores by increasing N concentrations in leaves (mechanism 4; Figure 1).

The aim of this study was to better understand the role of Si in anti-herbivore defences in a legume, particularly in relation to other defences (i.e. phenolics) and the JA pathway. We investigated the effects of both Si supplementation and JA application on four genotypes of lucerne (also known as alfalfa), *Medicago sativa*, an economically important temperate forage legume worldwide adapted to a wide range of climatic conditions (Baxevanos et al., 2022; Small, 2011). Si has the potential to benefit the lucerne–rhizobia interaction, increase lucerne resistance to chewing herbivores and improve lucerne health and productivity, with myriad ecological benefits (Putra et al., 2020). Four winter-active lucerne genotypes were tested to determine whether phytochemical and herbivore responses were consistent between genotypes. We quantified changes in foliar concentrations of Si, carbon (C), N and phenolics and determined how this affected feeding behaviour and growth rates of a chewing folivore (adult *Sitona discoideus* weevils). As summarised in Figure 1, we hypothesised that:

1. Si supplementation increases foliar concentrations of Si (silicification),
2. Foliar silicification alters concentrations of phenolics; both defences reduce feeding activity and performance of *S. discoideus*,
3. Stimulation of the JA pathway increases both Si and phenolic defences,
4. Si supplementation increases foliar concentrations of N which may moderate defences against *S. discoideus*.

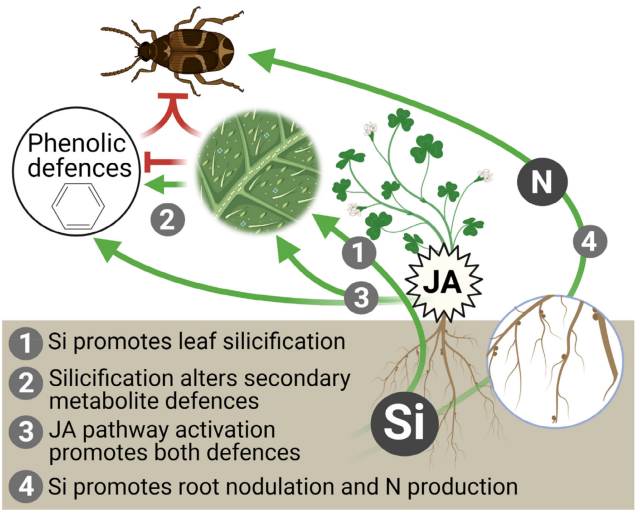

FIGURE 1 Conceptual model, based on the available literature, for how Si supplementation and jasmonic acid application may affect Si and phenolic defences against insect herbivores in legumes. Green arrows and red lines represent positive and negative effects respectively.

2 | MATERIALS AND METHODS

2.1 | Plant and insect material

Thirty-two seedlings of each of four lucerne genotypes (Aurora, Genesis, Sequel and Trifecta) were grown from seed (128 plants in total). All genotypes had similar growth patterns and their pest/disease resistance and yields are generally in the order of: Genesis > Aurora > Sequel ≥ Trifecta (Lowe et al., 2010; Ryalls et al., 2017; Williams & Young, 1996). Plants were grown in 70 mm width × 135 mm deep pots (allowing unrestricted growth for the duration of the experiment) filled with sieved (2 mm) local low-fertility loamy-sand soil collected from the Hawkesbury Forest Experiment in Richmond, NSW, Australia (see Ryalls, Moore, & Johnson, 2018 for chemical composition), which has a low bioavailable Si content of 10–17 mg kg^{-1} (Johnson et al., 2017). Plants were maintained in growth chambers at 26/18°C day/night on a 15L:9D cycle for 12 weeks (February–May 2017). High pressure sodium lights were used to maintain photosynthetic active radiation at 350 µmol m^{-2} s^{-1} during the day, which corresponded with levels recorded in glasshouse experiments in Richmond, NSW (Ryalls et al., 2013). Humidity was controlled at 60%. Half of the plants were supplemented with Si (500 mg L^{-1} solution of dissolved sodium metasilicate) and half received deionised water alone (*c.* 23 mL three times a week in both cases) without any amendment, as in Ryalls, Moore, Johnson, Connor, et al. (2018) and Johnson et al. (2017). The application of Si in the form of Na_2SiO_3 could slightly increase soil concentrations of sodium (Na), though treatment concentrations were very low (1 mM) and plants would most likely be unaffected as uptake of Na tends to decrease as plants take up Si (Kafi & Rahimi, 2011). Three days before the feeding assay, half of the Si-supplemented plants were treated with methyl jasmonate (MeJA; 95%, Sigma Aldrich) by applying 1 mL of a solution of 100 µg mL^{-1} MeJA in 0.1% Tween 20 to the base of the stem at the surface of the soil with a pipette, as in Johnson, Glauser, et al. (2018). The other half were supplemented with 1 mL of Tween 20 only. This was repeated 24 and 48 h later. One hundred and sixty sexually mature *S. discoideus* adults were collected from a local lucerne field in Richmond, NSW, Australia and maintained on propagated lucerne plants (40 weevils on each of the four genotypes) under the same conditions as the plants for 1 week prior to the feeding assay.

2.2 | Experimental procedure—Feeding assay

Four leaves were removed from each plant. The two youngest leaves remained on the plant and were not used. Two were weighed (using

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a 0.001 mg micro-balance) and placed in an empty Petri dish and the other two were weighed and placed in a Petri dish containing one adult weevil, which was starved for 24 h and weighed prior to the feeding assay (mean mass = 4.8 mg, ranging from 2.6 to 8.2 mg). After 24 h, remaining leaves were dried and reweighed. The dry mass eaten was calculated by subtracting the dry mass after feeding from the dry mass before feeding [i.e. fresh weight offered × (dry mass control at end/wet mass control at start)]. This was used to calculate the relative consumption (RC) for each adult weevil (Equation 1).

$$\text{RC (mg / mg)} = \frac{\text{dry mass eaten}}{\text{mean adult mass throughout herbivory period}} \quad (1)$$

Weevils were weighed after the feeding assay and starved for a further 12 h to ensure all frass was expelled, before being reweighed. Relative growth rate (RGR) was also defined for each adult weevil (Equation 2), as described by Johnson, Waterman, et al. (2021).

$$\text{RGR (mg/mg/day)} = \left(\frac{\text{Final adult mass after feeding} - \text{Initial adult mass before feeding}}{\text{Initial adult mass before feeding}}\right) \div \text{days} \quad (2)$$

Once the feeding assay had been initiated, remaining above-ground and below-ground plant parts were removed from the soil. Remaining soil was washed from plants over 200 and 20 μm stacked sieves and the numbers of nodules on the roots were counted. Roots were separated from shoots and all plant material was snap-frozen in liquid N before being freeze-dried and weighed.

2.3 | Chemical analyses

Dried leaf material was separated from stems and ball-milled to a fine powder prior to chemical analyses. Total Si concentrations (% of dry mass) were determined from all 128 plants with an X-ray fluorescence spectrometer (Epsilon 3 EDXRF, PANalytical, EA Almelo, The Netherlands, LLD = 1 ppm – 100%) using small mass holders (calibrated at 0%–10% using Si standards prepared by mixing SiO_2 with methyl cellulose and milling for 2 min at 30 Hz). The use of small mass holders minimises the quantity of material required to perform measurements (100 mg) without compromising the quality of the analysis, as described by Hiltpold et al. (2017). Si data were calibrated using citrus plant material of known Si concentration (NCS ZC73018 Citrus leaves 0.41%) for each run of nine samples. Carbon (C) and nitrogen (N) concentrations were determined for half of the leaf samples (64 plants; $N = 4$ per genotype per Si treatment per JA treatment) with a Carlo Erba CE1110 elemental analyser. Total phenolics were quantified from the other half of the plants ($N = 64$) using the Prussian blue assay and gallic acid as a standard (Graham, 1992). Approximately 10 mg of foliar tissue was extracted twice with 70% acetone (v:v) and the combined extracts were measured on a CLARIOstar High Performance Monochromator multimode microplate reader (BMG Labtech), following the protocol described by Waterman et al. (2021). Absorbance was averaged from three replicate extracts per sample and total phenolic concentration (TPC; mg GAE/g dry weight) was calculated using the formula TPC = cV/m, where c = concentration of gallic acid obtained from a calibration curve of known standards in mg/mL, V = volume of extract (0.5 mL) and m = mass of extract (0.05 g).

2.4 | Statistical analyses

The R statistical interface v4.2.0 was used for all statistical analyses. The effects of genotype, Si supplementation and JA application on plant growth (root mass, shoot mass and nodulation), foliar chemistry (%Si, %C, %N and total phenolics) and insect feeding metrics (RC, frass production and RGR) were analysed using general linear models. The fixed effects for all models included genotype (Aurora, Genesis, Sequel and Trifecta), Si treatment (Si− and Si+) and JA treatment (JA− and JA+) as well as the two-way and three-way interactions between these terms. Post-hoc tests using the package *emmeans* were used for pairwise comparisons of means. Where appropriate, dependent variables were transformed before analysis (Table 1). Pearson's correlations were used to determine whether foliar Si and phenolic concentrations were associated with RC, frass production or RGR.

3 | RESULTS

3.1 | Plant growth

Si supplementation significantly increased shoot mass (Figure 2A), root mass (Figure 2A) and the number of root nodules (Figure 2B). Full statistical results are shown in Table 1. Root mass, shoot mass and nodulation tended to be lowest in the genotype Aurora compared with the three other genotypes (Figure 2). No significant effects of JA treatment, and no interactions between fixed effects (Figure S1) were observed.

3.2 | Foliar chemistry

Si supplementation significantly increased foliar Si (Figure 3a) and C concentrations (Figure 3b). JA treatment also increased the concentration of foliar C (Figure 3b). Foliar N concentrations were not affected by Si or JA treatments (Figure 3c), individually or in combination. Si treatment in interaction with JA treatment significantly affected total phenolics, which increased when JA was applied, especially when plants were supplemented with Si (Figure 3d). Genotype had no significant effect on any of the foliar chemicals measured (Table 1; Figure S2).

3.3 | Weevil feeding metrics

Si supplementation significantly decreased both the RC of leaf material (Figure 4a) and the amount of frass produced (Figure 4b). While

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TABLE 1 Plant growth, foliar chemistry and weevil responses to plant genotype, Si treatment (silicon) and jasmonic acid (JA) treatment from linear models.

			Genotype		Silicon		JA		Genotype × silicon		Genotype × JA		Silicon × JA		Genotype × silicon × JA	
Response variable	**Fig.**	**df**	F/χ^2_3	***p***	F/χ^2_1	***p***	F/χ^2_1	***p***	F/χ^2_3	***p***	F/χ^2_3	***p***	F/χ^2_1	***p***	F/χ^2_3	***p***
Plant growth																
Shoot mass (mg)[a]	2A	112	3.47	**0.019**	17.68	**<0.001**	0.36	0.55	0.98	0.404	0.58	0.628	0.11	0.741	0.16	0.921
Root mass (mg)[a]	2A	112	10.76	**<0.001**	29.82	**<0.001**	2.29	0.133	1.26	0.293	0.33	0.802	0.06	0.803	0.75	0.526
Nodulation (#)	2B	112	8.12	**0.044**	22.89	**<0.001**	0.01	0.903	4.13	0.248	0.33	0.954	1.72	0.190	3.51	0.320
Foliar chemistry																
Silicon (%)[a]	3A	112	0.40	0.758	14.75	**<0.001**	0.62	0.433	0.16	0.925	0.55	0.646	0.03	0.870	0.35	0.791
Carbon (%)	3B	48	0.68	0.570	4.44	**0.040**	6.73	**0.013**	1.25	0.303	0.72	0.543	0.14	0.708	1.42	0.248
Nitrogen (%)	3C	48	1.71	0.178	0.03	0.860	0.95	0.333	0.49	0.690	0.29	0.835	0.51	0.480	0.05	0.986
TPC (mg/g DW)	3D	48	0.86	0.469	4.06	**0.049**	39.39	**<0.001**	0.43	0.734	0.04	0.990	4.96	**0.031**	0.05	0.987
Weevil feeding metrics																
RC (mg/mg)	4A	111	2.36	0.076	15.96	**<0.001**	12.16	**<0.001**	1.11	0.347	0.14	0.934	0.34	0.562	0.10	0.959
Frass (mg)[a]	4B	110	1.35	0.261	12.98	**<0.001**	1.85	0.176	0.14	0.937	0.61	0.613	0.05	0.822	0.40	0.754
RGR (mg/mg/day)	S1	111	3.42	**0.020**	1.05	0.307	0.91	0.343	0.70	0.554	1.81	0.149	1.38	0.243	2.01	0.117

Note: *p*-values highlighted in bold indicate significance ($p < 0.05$). Where appropriate, response variables were transformed ([a]square root) before analysis. TPC, RC and RGR refer to total phenolic concentration, relative consumption and relative growth rate respectively. Nodulation (negative binomial model) is reported as a likelihood-ratio χ^2 test statistic. All other parameters (general linear models) are reported as *F*-values.

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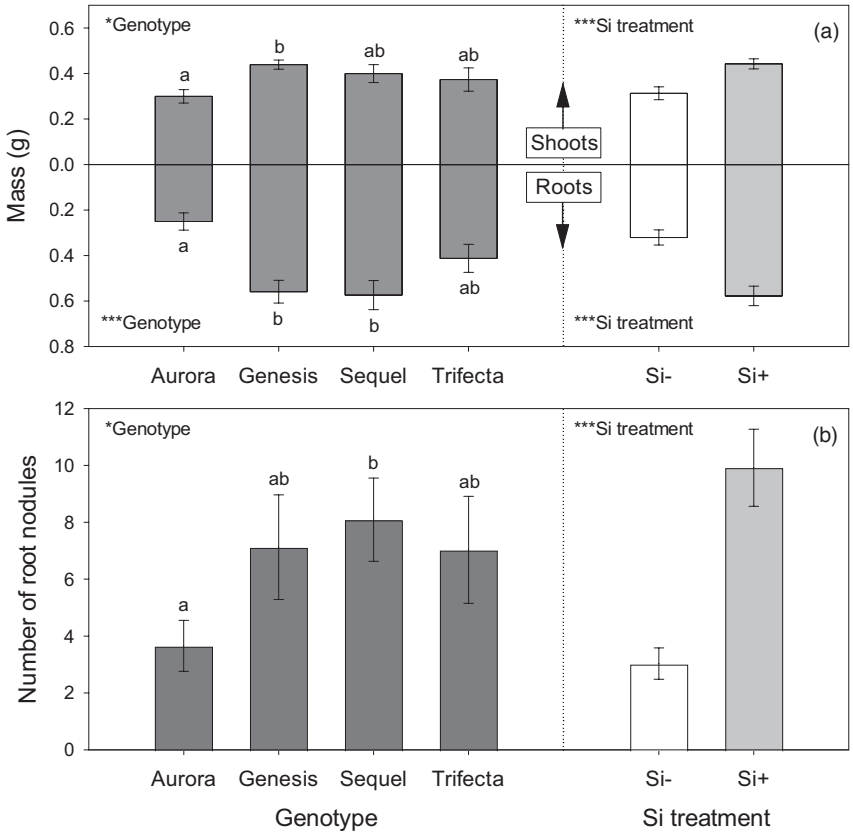

FIGURE 2 The effects of genotype and silicon (Si) supplementation on the shoot and root mass (A) and root nodulation (B) of *Medicago sativa* plants. Values are means ± SE. Bars with the same letters were not significantly different ($p < 0.05$). Statistical significance of fixed effects indicated by *($0.01 < p < 0.05$) and ***($p < 0.001$); other individual fixed effects or two-/three-way interactions were not significant ($p < 0.05$).

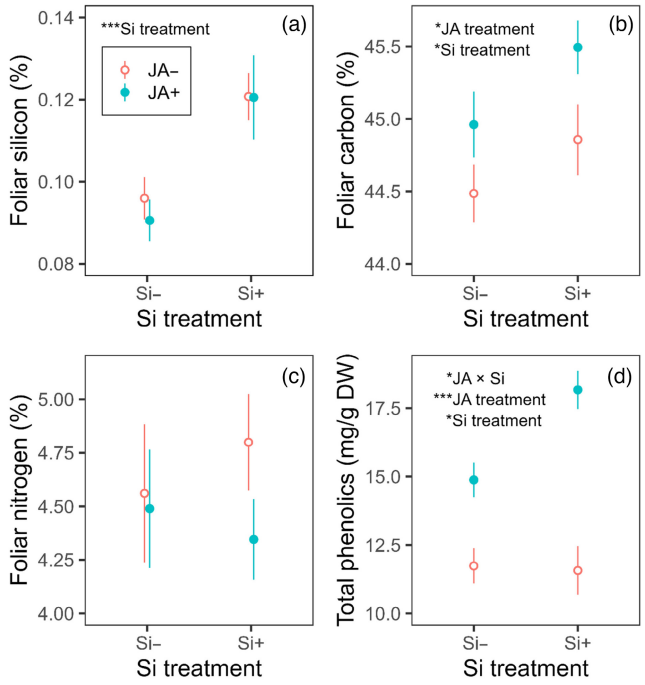

FIGURE 3 The effects of jasmonic acid (JA) application and silicon (Si) supplementation on foliar silicon (a), carbon (b), nitrogen (c) and total phenolic concentrations (d) of *Medicago sativa*. Values are means ± SE. Statistical significance of fixed effects indicated by *($0.01 < p < 0.05$) and ***($p < 0.001$); other individual fixed effects or two-/three-way interactions were not significant ($p < 0.05$).

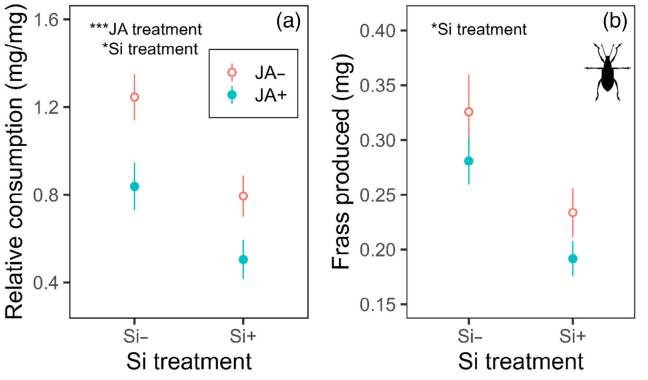

FIGURE 4 The effects of jasmonic acid (JA) application and silicon (Si) supplementation on relative consumption (a) and frass production (b) by *Sitona discoideus*. Values are means ± SE. Statistical significance of fixed effects indicated by *($0.01 < p < 0.05$) and ***($p < 0.001$); other individual fixed effects or two-/three-way interactions were not significant ($p < 0.05$). Weevil image by JCGiron using the R package *phylopic*.

JA application significantly decreased RC (Figure 4a), frass production was not significantly lower when JA was applied (Figure 4b). No genotype effect, two-way or three-way interactive effects on RC or frass production were observed (Table 1; Figure S4). Herbivore RGR was not significantly affected by JA or Si treatments, individually or interactively. However, RGR was significantly higher in the genotype Genesis compared with Sequel (Figure S3). Total phenolic and silicon

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concentrations were significantly correlated with RC (increasing phenolics or silicon decreased RC; phenolics: $r=-0.259$, $p=0.041$, df = 61, silicon $r=-0.232$, $p=0.009$, $df=125$), but not frass production (phenolics: $r=-0.200$, $p=0.116$, df = 61, silicon: $r=-0.052$, $p=0.565$, df = 125) or RGR (phenolics: $r=-0.001$, $p=0.974$, df = 61, silicon: $r=0.053$, $p=0.558$, df = 125; Figure S5).

4 | DISCUSSION

4.1 | Si supplementation increased foliar Si and plant growth in lucerne

Most studies on biotic responses to plant Si deposition have used species of Poaceae (mostly cereal crops) as high Si-accumulating model plants (Cooke & Leishman, 2016; Johnson, Hartley, et al., 2021), in which Si is assumed to be more functionally important. Foliar Si concentrations in legumes have been shown to increase (Nascimento et al., 2014), decrease (Johnson, Ryalls, et al., 2018) or remain unchanged (Johnson et al., 2017) in response to Si supplementation and the outcome may depend on the diversion of resources towards plant growth (e.g. silicification may reduce the high metabolic cost of C-based compounds in cell wall construction; Putra et al., 2022). Plant growth rates over 12 weeks, and Si-associated increases in above- and below-ground mass, were consistent with previous studies with *M. sativa* (Johnson et al., 2017; Johnson, Ryalls, et al., 2018; Ryalls et al., 2015). Studies with other legumes have also demonstrated increases in plant mass in response to Si supplementation (Guérin et al., 2014; Rasoolizadeh et al., 2018). Foliar Si concentrations increased in response to Si supplementation despite the increase in plant growth, which may explain why Si supplementation decreased the relative consumption of foliage by *S. discoideus* weevils.

4.2 | Si and JA supplementation stimulated phenolic production and reduced herbivore feeding

While previous studies have identified potential trade-offs between foliar Si and C-based defences such as phenolics (Cooke & Leishman, 2012; de Tombeur et al., 2021; Johnson & Hartley, 2018), we observed the opposite in *M. sativa* in this current study. Other studies have suggested that Si potentially optimises other plant defences as part of a wider herbivore defensive syndrome (Cibils-Stewart et al., 2022; Moles et al., 2013). Si deposits in tissues may exacerbate the physical aggravation caused by herbivore damage to increase JA activity and downstream production of secondary metabolite defences (Hall et al., 2019). This may therefore explain why Si supplementation promoted the production of phenolics when JA was applied, since stimulation with MeJA simulates herbivore attack. In the absence of this stimulation, foliar phenolic concentrations were largely unchanged by Si supplementation. Studies measuring individual phenolics, which represent a broad family of compounds with many roles, with greater focus on the distribution of silicon and phenolic compounds at the cellular level, would provide further mechanistic insights into how legumes and other plants prioritise Si transport among different above- and below-ground tissues (Hodson & Guppy, 2022).

We observed a JA-induced reduction in relative consumption by *S. discoideus* weevils, which was associated with an increase in phenolics. Phenolics and relative consumption were negatively correlated, although this trend was not reflected in the short-term growth of *S. discoideus*. The application of JA has also promoted phenolic production in other plant species, such as *Catharanthus roseus* (Liu et al., 2016), *Brassica oleracea* (Guan et al., 2019), *Ocimum basilicum* (Malekpoor et al., 2016) and *Picea abies*; this latter example been associated with resistance to the pine weevil, *Hylobius abietis* (Puentes et al., 2021). While consuming less food tends to decrease herbivore frass production, decreased digestibility of food can counteract this (Massey & Hartley, 2009), which may explain why JA supplementation and phenolic production did not significantly alter the frass production of *S. discoideus*. Other physical defences, such as changes in leaf hair density, may also have a role to play in the observed feeding responses by *S. discoideus*. For example, Si and JA induction have been shown to reduce the ingestion of plant material by chewing herbivores by increasing the density of leaf macrohairs (Johnson, Hartley, et al., 2021). In general, feeding trials demonstrating changes in the relative consumption of leaf material could result from physical changes in plant tissues but can also result from changes in nutritional quality. In the current study, the relative consumption of leaf material was higher when lucerne was not supplemented with Si or JA, which is unlikely to be a compensatory feeding response (resulting from reduced plant nutrition that is suboptimal to the herbivore) because leaf nitrogen concentrations remained unchanged (Moise et al., 2019).

4.3 | Si increased root nodulation but not foliar N concentrations

Root nodule abundance increased in response to Si supplementation, which has previously been demonstrated in *M. sativa* (Johnson et al., 2017; Johnson, Ryalls, et al., 2018) and *M. truncatula* (Putra et al., 2022). This may have been a compensatory response to higher plant growth to prevent the plant from becoming N limited. Alternatively, Si-induced root nodulation may have provided the N, allowing for increased growth. While the exact mechanisms of why Si increases nodulation and benefits the legume–rhizobia interaction are unclear, it has been suggested that Si optimises solute transport and gas exchange in root nodules. Moreover, the lower metabolic cost of Si in cell wall construction may further increase nodulation. However, this Si-induced nodulation response was not reflected in foliar N concentrations. Studies using grass species tend to show negative effects of Si supplementation on foliar N concentrations (Johnson, Waterman, et al., 2021), whereas this relationship is likely to be less apparent in legumes, which only accumulate

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low to moderate levels of Si (Putra et al., 2021). Si has been shown to promote foliar N and N-containing compounds in *Medicago* spp. (Johnson, Ryalls, et al., 2018; Putra et al., 2022). In this study, however, increased plant growth may have caused a dilution effect, whereby increased uptake of foliar N was accompanied by greater tissue mass. Moreover, root Si deposition may anatomically alter the structure of root nodules but does not necessarily promote rhizobial N fixation (Putra et al., 2021) and foliar N concentrations may be dictated by rhizobial efficacy. For example, when low-efficiency rhizobial strains were present in *M. truncatula*, foliar N was found to increase, whereas plants with high-efficiency strains had no effect on foliar N concentrations (Putra et al., 2022). Experimental studies such as this would benefit from comparisons with feeding trials using field-grown plants prior to flowering (to minimise variation associated with resource allocation to flowering). Controlled field experiments with and without plots with Si and/or JA supplementation would help to determine more field-realistic changes in phytochemistry and the potential for field applications of Si to assess its anti-herbivore benefits and soil N inputs on a wider scale.

5 | CONCLUSIONS

Given the importance of N fixation in legumes to ecosystem function and the potential for Si to positively impact the symbiosis between legumes and rhizobia bacteria in root nodules, surprisingly few studies have examined the effects of Si on legumes. Si clearly has the potential to increase nodulation and plant growth of legumes, while reducing herbivore feeding. Moreover, when Si is combined with an exogenous application of JA, it has the potential to stimulate additional anti-herbivore defence. The observed Si- and JA-induced phytochemical and herbivore responses were consistent between four lucerne genotypes. As such, the field of insect–plant ecology would benefit from further understanding into Si dynamics in legumes and their responses to JA application to enhance crop protection and ecosystem productivity.

AUTHOR CONTRIBUTIONS

J. M. W. Ryalls conceived the experimental design, carried out data collection, carried out data analyses and drafted the manuscript. A. N. Gherlenda participated in data collection. R. C. Rowe performed phenolic analysis. B. D. Moore participated in the design of the study. S. N. Johnson participated in the design of the study and in drafting the manuscript. All authors provided feedback on the final manuscript draft and gave approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.t4b8gtj70 (Ryalls et al., 2023).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. The effects of jasmonic acid (JA) application and silicon (Si) supplementation on shoot mass (a), root mass (b) and root nodulation (c) of four genotypes of *Medicago sativa*. Values are means ± SE.

Figure S2. The effects of jasmonic acid (JA) application and silicon (Si) supplementation on foliar silicon (a), carbon (b), nitrogen (c) and total phenolic concentrations of four genotypes of *Medicago sativa*. Values are means ± SE.

Figure S3. *Sitona discoideus* relative growth rate (RGR) changes between different genotypes of *Medicago sativa* (a) and the three-way interactive effects of silicon (Si), jasmonic acid (JA) and plant genotype on *S. discoideus* RGR. Values are means ± SE. Only plant genotype had a statistically significant effect on RGR (a); bars with the same letters were not significantly different ($p < 0.05$).

Figure S4. The effects of jasmonic acid (JA) application and silicon (Si) supplementation on relative consumption (a) and frass production (b) by *Sitona discoideus* weevils fed on four genotypes of *Medicago sativa*. Values are means ± SE.

Figure S5. Correlations between foliar silicon (a, c, e) or total phenolics (b, d, f) and *Sitona discoideus* weevil metrics (relative consumption or RC, frass produced, relative growth rate or RGR). Data shown and in-text statistical results are correlations between raw (untransformed) variables. Variable transformation had no effect on significance. Trendlines with means ± 95% confidence intervals shown for significant correlations only.

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