Drought and endophyte impact African black beetle feeding on perennial ryegrass

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Abstract

Water is essential to plant growth, driving interactions between plants, herbivorous insects and fungal *Epichloë* endophytes. However, water availability fluctuates, a phenomenon intensified by climate change, challenging the success of New Zealand's pastoral industry. The impacts of climatic changes, such as intensifying drought, on pastoral insect pests have received little attention. In a no-choice bioassay, African black beetle (ABB) were fed semi-synthetic diets containing freeze-dried foliage material from drought-exposed and well-watered perennial ryegrass containing endophyte strains AR37 or NZ_{CT}, or with material free of endophyte (Nil). Diet consumption, beetle weight change, fungal alkaloid concentrations, phytohormones, and NRI (nitrogen reflectance index) concentrations were measured and compared. Except for AR37, ABB consumed more diets containing drought-exposed rather than well-watered material, with a 61% increase in Nil and a 50% increase in diets containing NZ_{CT}, though these effects were not accompanied by corresponding ABB weight changes. Here, we discuss the implications of these results on ABB damage in New Zealand pastures in the context of increasing drought and illustrate how spatial patterns of ABB damage could change over the next 75 years. Areas that do not currently have high populations of ABB such as Manawatū-Whanganui, Wairarapa and Marlborough will become increasingly vulnerable to damage.

Keywords: *Epichloë*, climate change, pasture resilience, alkaloids, insect feeding, *Heteronychus arator*, *Lolium perenne*

Introduction

The success of New Zealand's pastoral industry is closely linked to its mild and moist temperate climate allowing year-round grazing. Water deficit can be a major limiting factor for pasture growth and production, especially in perennial ryegrass swards (*Lolium perenne* L.). Climate change increases the occurrence and intensity of drought which will challenge modern pasture systems. In New Zealand, climate predictions are highly variable by region, although, the predicted overall trend is an increased likelihood of soil moisture depletion in northern and eastern regions in the North Island and northeast parts of the South Island, with an increase in rainfall in the remaining areas (Sood and Mullan 2020). Under extreme drought projections, New Zealand's time in drought will increase by over 10% in all regions, except the West Coast of the South Island (NIWA 2011). With such changes, insect pest populations are predicted to change and hence their impacts on pasture production are also expected to change (White 1969; Huberty and Denno 2004; Lin et al. 2022). For example, drought can lead to major pest outbreaks in the following year if conditions are more favourable for the pest or/and unfavourable for natural enemies (Kalmakoff et al. 1985; Schreven et al. 2017; Mansfield et al. 2021; Lin et al. 2022). In New Zealand, this can occur for porina (*Wiseana* spp.) and grass grub (*Costelytra zealandica*) where outbreaks are observed 2-4 years after a severe drought (East and Willoughby 1980). This is because drought also kills natural insect pathogens and these are slower to recover from extreme weather events than the insect pest themselves, leading to low pathogen infection rates in the next few generations (O'Callaghan 1989). Similarly, African black beetle (*Heteronychus arator* Fabricius, ABB) outbreaks have been linked with La Niña events (Gerard et al. 2013).

Originally introduced from South Africa, ABB is the second most economically damaging pest to pastures and arable crops in New Zealand (Ferguson et al. 2019). Adults feed at the crown of pasture plants, while larvae feed on the roots. ABB was previously confined to Northland and Auckland. However, in the last 90 years, its distribution has extended to the Waikato, East Cape, Gisborne areas, Hawkes Bay and Manawatū, and continues to gradually extend south (Watson 1978; Bell et al. 2011; Mansfield et al. 2021). ABB larvae are particularly problematic during the dry summer months

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because they feed on the roots close to the soil surface causing severe damage at a time when plants are already drought-stressed, which can lead to plant death.

There are few control options for ABB (Karpyn Esqueda et al. 2017; Mansfield et al. 2019), one being the use of asexual fungal *Epichloë* endophytes. These mutualistic, seed-transmitted fungi have become an essential component of pasture persistence by mitigating the effects of biotic and abiotic challenges (Caradus 2023). Some strains of the fungal endophyte *Epichloë* in perennial ryegrass can reduce adult ABB damage leading to greater resilience under drought, contributing to higher persistency than endophyte-free plants (Ball et al. 1992). Commercially available endophyte strains such as AR37, NZ_{CT}, NEA2, and NEA4 in perennial ryegrass reduce adult ABB populations and hence damage in the field, majorly contributing to pasture growth in comparison to endophyte-free plants (Hume et al. 2007; Thom et al. 2013; Stewart et al. 2014). Similarly, endophyte strain MaxP in tall fescue (*Festuca arundinacea* L.) reduces damage from beetle adults and larvae (Stewart et al. 2014). The success of *Epichloë* endophytes protecting its host grass is linked to the production of fungal alkaloids. Ergovaline has a strong antifeeding effect on adult ABB reducing oviposition and plant damage (Ball et al. 1997; Thom et al. 2013). While ergovaline only deters adult ABB, the fungal alkaloid loline produced by the tall fescue endophyte *Epichloë coenophiala* also reduces larval growth (Bryant et al. 2010; Barker et al. 2015).

Interactions between insect feeding damage and plant water availability in pastures are poorly understood though it is this combined pressure that often causes plant death (Tozer et al. 2017; Hewitt et al. 2021). This paper examines how drought influences plant-ABB interactions in endophyte-infected pastures and makes a preliminary evaluation of how climate change could influence the distribution and impact of ABB in New Zealand pastures. The objectives of this study were to

(i) Evaluate in a bioassay if drought increases adult ABB feeding in *Epichloë* endophyte-infected perennial ryegrass.
(ii) Model ABB's potential distribution in New Zealand under climate change while also considering changes in drought susceptibility.
(iii) Draw conclusions about the ability of endophytes to provide protection against ABB in a changing environment.

Materials and Methods

Bioassay

In a no-choice bioassay, ABB were fed with semi-synthetic diets containing freeze-dried foliage material from drought-exposed and well-watered perennial ryegrass plants containing endophyte strains AR37 (*Epichloë* LpTG-3) or NZ_{CT} (*Epichloë festucae* var. *lolii*), or material free of *Epichloë* endophyte (Nil). Diet consumption and beetle weight change were assessed. The freeze-dried plant material used to create semi-synthetic diets was analysed and compared for their fungal alkaloid concentrations, phytohormones, and nitrogen reflectance index (NRI).

Establishment of ryegrass plants for bioassay

In a glasshouse diploid perennial ryegrass seeds (cv. One50) were sown in a standard seed-raising mix (Daltons™). At the 6-tiller stage, plants were tested for viable endophyte infection using the tissue immuno-blot technique (Simpson et al. 2012). Plants were transplanted into 100 cm long, 8 cm diameter PVC tubes filled with soil substrates. Each tube's weight was recorded enabling the calculation of percent soil moisture per tube during the experiment. A further set of eight tubes without plants was saturated with water, and the soil was oven dried at 80°C enabling the calculation of field capacity and permanent wilting point of each tube. Further details of the tube set-up are described in Hewitt et al. (2023). Plants were kept at 1% above the permanent wilting point for i) 8 weeks, ii) 9 weeks, and iii) 13 weeks in which plant material was collected. Well-watered plants were watered as required to meet 80% field capacity. At harvest, plants were trimmed to 2 cm above soil level using sterilised scissors (dipped into 70% ethanol between plants to avoid cross contamination of potential plant diseases) and immediately frozen to -20°C for later freeze drying. Plants were bulked according to treatment combinations (Table 1). This was done to create sufficient plant material to prepare semi-artificial insect diets.

Diet preparation

Fresh, organically-grown carrot (750 g) was blended with 1.5 L MilliQ water and strained to obtain 600 mL carrot juice before adding 14.4 g of agar. To prevent fungal growth in the diet 0.048 g streptomycin and 0.048 g penicillin was added to the agar-carrot and thoroughly mixed. The mixture was heated in a microwave until boiling point. The preservatives methyl parahydroxybenzoate (0.0169 g) and sorbic acid (0.141 g) were added once the mixture cooled to 70°C. Eight diet batches (27 g) were weighed out separately into warm glass beakers and 3 g of freeze-dried, ground foliage was added to each beaker, thoroughly mixed, and poured into 9 cm diameter Petri dishes. Petri dishes were wrapped in tin foil to minimise alkaloid compound degradation from the light and placed in the fridge at 4°C.

Black beetle collection

Adult ABB were collected from pitfall traps set in an established pasture on a Waikato dairy farm over 2 weeks in spring (September 2021). Beetles were separated by gender and placed into groups of 20 in containers filled with moist soil and fed every 3 days with fresh organic carrots until the start of the bioassay.

Table 1 Number of bulked perennial ryegrass plants free of *Epichloë* (Nil) or infected with *Epichloë* AR37 or NZ_{CT} used to create semi-artificial African black beetle diets. Drought-exposed plants were kept at 1% above permanent wilting point for 8, 9, and 13 weeks. Diets were created from the total bulked number of plants for each treatment combination.

	Number of plants (n)			
Drought duration	**8 weeks**	**9 weeks**	**13 weeks**	**total**
Drought				
Nil	49	44	44	137
AR37	37	32	32	101
NZ_{CT}	35	30	30	95
Well-watered				
Nil	35	30	30	95
AR37	37	32	32	101
NZ_{CT}	35	30	30	95

Bioassay set-up

The effect of drought on ABB was assessed by comparing diet consumption and beetle weight change when fed diets containing different endophyte strains and endophyte-free plants and drought versus well-watered plants. A 2 weeklong no-choice bioassay was conducted using adult ABB in individual 60 mL plastic containers containing damp wood shavings. There were 16 replicate beetles (8 male, 8 female) for each treatment combination. ABB were starved for 24 h and weighed prior to the bioassay. Each ABB was provided with a 14 mm diam. diet plug containing ground plant material from one of the treatments. Each diet plug was placed in a 2.5 ml vial laid sideways in the container to allow easy access to the diet for the beetle. Fresh diets were made weekly and diet plugs were changed on day 4 and day 7 of each week. Diets were kept at 4°C between diet plug changes. Diet consumption was measured by the change in diet weight between diet changes. Beetle survival was checked at each diet change and again after 2 weeks at the completion of the bioassay. The bioassay was conducted in a controlled temperature room set to 20°C with a 16:8 h light:dark regime.

Chemical analyses

Fungal alkaloid concentrations, phytohormones, and NRI were quantified on bulked freeze-dried ground foliage material. There were three technical replications per treatment combination (repeated measurement of the same sample). Fungal alkaloids peramine, lolitrem B, ergovaline, and epoxyjanthitrems were analysed using the methods described in Miller et al. (2022). NRI was determined at Hill Laboratories. Phytohormone analysis was performed at AgResearch (W. Mace, unpublished).

Statistical analyses

Analysis of variance (ANOVA) was carried out on diet consumption and beetle weight change using "moisture" (drought and well-watered) and endophyte infection (Nil, AR37, NZ_{CT}) as treatment factors. ANOVA residuals were checked for homogeneity of variances. Fisher's least significance difference tests were used to assess the significance of the relationship between diet consumption and moisture stress at α=0.05. For a graphical presentation of the data and to confirm the impact of fungal alkaloids, plant hormones, and nutritional status of the plant on ABB diet consumption and beetle weight gain/loss we performed a principal component analysis (PCA). Values from the nutritional, alkaloid, and plant hormones represent means of bulked material. PCA was carried out using the Data integration app (Luo 2022). All graphs were generated using SigmaPlot 14.0 (Systat Software Inc.).

Climate modelling

Data processing, modelling, and graphing was conducted using R version 4.2.3 (R Core Team 2013) and functions from packages including the tidyverse (Wickham et al. 2019), SPEI (Beguería et al. 2014), sf (Pebesma 2018) and snowfall (Knaus 2022).

Climate data

Historical climate data for New Zealand were NIWA's Virtual Climate Station Network (VCSN) data (NIWA 2023) comprising daily rainfall and maximum and minimum air temperatures for 1991-2010 ("2000") on a regular 0.05° grid (~5 km) of 11491 locations. Future climate projections for the periods 2041-2060 ("2050") and 2080-2099 ("2090") comprised the same variables at the same 0.05° resolution and were also obtained from NIWA (Mullan et al. 2018). The future projections were from the Hadley Centre Global Environment Model version 2 (HADGEM2) based on Representative Concentration Pathway (RCP) 6.0. The VCSN data contained 40 more grid cells than the future climate projections (n = 11451) thus these were removed so the VCSN and future climate grids perfectly matched.

Black beetle distribution

Watson (1978) observed that in New Zealand black beetle occurred in locations with mean annual air temperatures >12.8 °C and this relationship has persisted as New Zealand's climate has warmed, and ABB has spread southwards (Bell et al. 2011; Mansfield et al. 2021). We used Watson's (1978) simple model to estimate ABB potential New Zealand distribution for the 2000, 2050 and 2090 periods by calculating the mean of the maximum and minimum air temperatures and identifying grid cells where the mean was >12.8 °C.

Drought index

The Standardised Precipitation Evapotranspiration Index (SPEI) calculates, for a given location, precipitation minus potential evapotranspiration, then gives the number of standard deviations the transformed value differs from the mean value for the same location during a specified reference period (Vicente-Serrano et al. 2010). It is used by NIWA and StatsNZ for reporting drought in New Zealand (StatsNZ 2020). To calculate the SPEI, climate data for the 2000, 2050 and 2090 periods were summarised to monthly values, and functions from R package 'SPEI' (Beguería et al. 2014) were used to calculate potential evapotranspiration (based on the equation of Thornthwaite (1948)) and SPEIs for each month (n = 12 months x 20 years = 240) in each period (n = 3) in each grid cell (n = 11451). SPEIs were calculated using a time scale of 6 months, which is generally regarded as corresponding with agricultural drought (StatsNZ 2020) and the 2000 period was used as the reference to which the 2050 and 2090 periods were compared. Using a time scale of 6 months meant no SPEI could be calculated for the first 5 months of each period, thus SPEIs were obtained for 235 months per period per grid cell. A location with a SPEI ≤ 1 is regarded as experiencing at least moderate drought (Kingston and Treadwell 2020). Thus, to summarise drought frequency and magnitude at each grid cell in each period, monthly SPEIs ≤ 1 at each grid cell were summed.

Results

Bioassay

Endophyte and moisture had significant effects on ABB diet consumption. ABB generally fed more on drought-exposed than well-watered diets (moisture P<0.001). Beetles also fed significantly more on diets containing Nil perennial ryegrass than on AR37 and NZ_{CT} (Nil>AR37>NZ_{CT}; endophyte P<0.01). In droughted material, Nil had greater feeding than AR37 and NZ_{CT} (P<0.005), with AR37 and NZ_{CT} being similar (P>0.05, Figure 1). In an interaction between endophyte and moisture, ABB consumed more diet containing drought-exposed rather than well-watered material for Nil (+61%) and NZ_{CT} (+50%) but moisture status in diets did not affect consumption for AR37. In diets containing well-watered plant material, NZ_{CT} reduced ABB feeding the most (P<0.01).

Chemical analyses

Peramine, ergovaline and lolitrem B were only detected in foliage infected with NZ_{CT}. Although no statistical analysis could be performed due to the three technical replications per treatment combination, the measured values suggested that peramine and lolitrem B concentrations were 7% lower in diets containing drought-exposed rather than well-watered plant material (Table 2). In contrast, drought may have increased ergovaline concentration by 42% in NZ_{CT}-infected plants and total epoxyjanthitrems concentration in AR37-infected plants by 52%, compared to well-watered plants (Table 2).

ABB generally lost weight during the 2-week bioassay. Beetles feeding on diets containing AR37 and NZ_{CT} lost significantly more weight than beetles feeding on diets containing endophyte-free (Nil) plant material (P<0.01). Plant moisture status did not affect ABB weight change (Figure 2) (P>0.05).

Relationship between drought, plant, and African black beetle

The PCA correlation structure of the traits is indicated by the directional vectors in the biplot (Figure 3). The first (PC1) and second (PC2) principal components of the PCA explained 81.6% (PC1= 64.7%; PC2=16.9%) of the variation in the data. PC1 separated the water treatments: well-watered treatments (rectangles) were on the positive side of PC1, and the drought treatments (circles) on the negative side. This signified PC1 as the "drought axis", characterised by a number of carbon- and nitrogen-containing traits, plant growth regulators such as IAA (indole-3-acetic acid) and epoxyjanthitrems in the negative direction of PC1. This contrasted with the fibre ash, lignin, and C:N ratio which all increased in the positive (well-watered) direction.

Both Nil endophyte treatments were found on the positive side of PC2, and the well-watered NZ_{CT} treatment on the negative PC2 side. The latter was characterised by several endophyte alkaloids, in the form of lolitrem B, peramine and ergovaline contributing to beetle weight loss (Figure 3). PC2 can therefore be termed the "endophyte axis". Opposite to these traits were diet consumption and crude fat on the positive side of PC2.

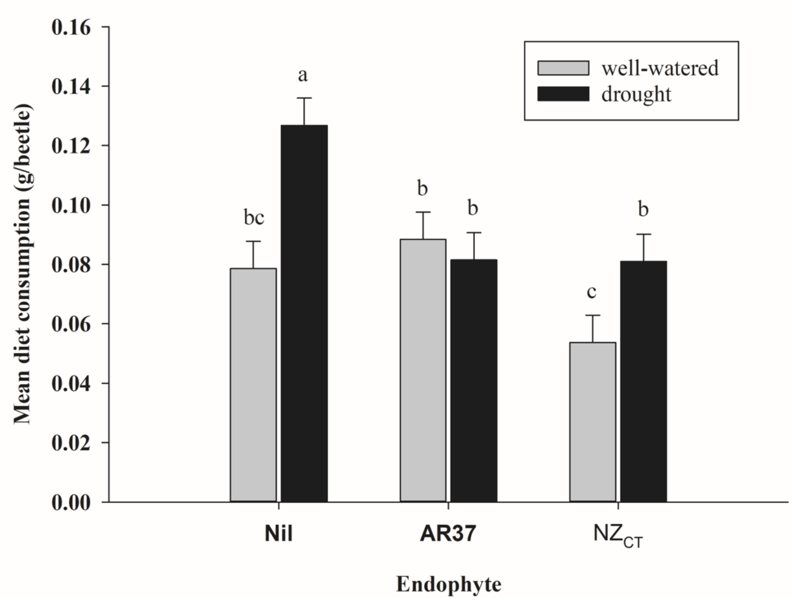

Figure 1 African black beetle consumption of diets (g/beetle) containing drought-exposed and well-watered perennial ryegrass plant material free of *Epichloë* (Nil) or infected with *Epichloë* strains AR37 or NZ_{CT}. Error bars represent standard errors of the mean (+ 1 SEM). Values with the same letter compare means between endophyte strains and moisture status and are not significantly different at P<0.05.

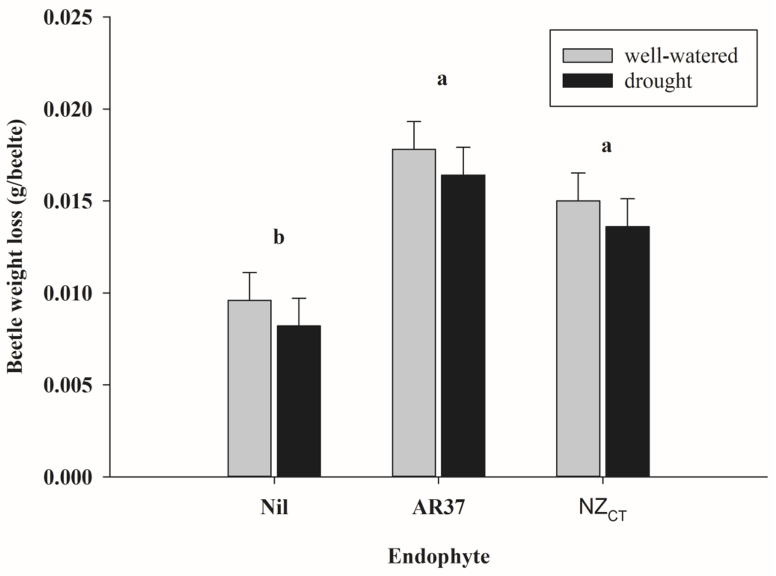

Figure 2 African black beetle weight loss (g/beetle) after feeding on diets containing well-watered and drought-exposed perennial ryegrass free of *Epichloë* (Nil) or infected with *Epichloë* strains AR37 or NZ_{CT} for 2 weeks. Error bars represent standard errors of the mean (+ 1 SEM). Values with the same letter compare means between endophyte strains and are not significantly different at P<0.05.

Table 2 Peramine, ergovaline, and lolitrem B concentrations (mg/kg) for perennial ryegrass infected with *Epichloë* strain NZ_{CT}, and total epoxyjanthitrems concentration (mg/kg) for perennial ryegrass infected with *Epichloë* strain AR37, for watered and drought-exposed plants. Values represent the mean of three technical replications which were measured in the bulked plant material consisting of 95-137 plants (Table 1).

	Alkaloid (mg/kg)			
Drought duration	**Peramine**	**Ergovaline**	**Lolitrem B**	**Epoxyjanthitrem**
Well-watered	38.8	0.7	5.1	108
Drought	36.1	1.0	4.7	165

Climate modelling

As expected, the area of New Zealand predicted to experience mean annual temperatures > 12.8 °C notably increased between the 2000 and 2090 periods, thus suggesting ABB's distribution in New Zealand will also continue to expand (Figure 4). In 2000, 2104 (18 %) of the 11451 grid cells spanning New Zealand had temperatures > 12.8°C, whereas in 2050 this increased to 4207 (37 %) and in 2090 to 4988 (44 %) (Figure 4). For grid cells with temperatures > 12.8 °C, the sum of SPEI ≤ 1 in each period (n = 235 months per period) varied between periods. As expected for the reference period, 2000, the sums were normally distributed (minimum -69, median -57, mean -57, maximum -46). In contrast, sums for 2050 were left-skewed (minimum -501, median -82, mean -92, maximum -1), as were those for 2090 (minimum -487, median -95, mean -102, maximum -1). Only 1 % of values for 2050 (n = 4207) and 2090 (n = 4988) were less than -300 so to simplify presentation they were binned with values up to -300 (Figure 4). The overall decrease in sum of SPEI ≤ 1 between 2000 and 2090 indicated that the duration and/or degree of precipitation deficits generally increased with period. This suggests that as ABB's potential distribution in New Zealand increases between 2000 and 2090, so will the duration and degree of precipitation deficits (Figure 4), thus potentially compounding ABB damage to pasture. Some of the North Island regions that could become increasingly vulnerable to ABB damage due to warming and drought include Waikato, coastal Bay of Plenty, and parts of Taranaki, Manawatū-Whanganui and Wairarapa (Figure 4). By 2090, some coastal parts of Canterbury, Marlborough, Nelson, Tasman, and West Coast are also predicted to become warm enough for ABB, though the SPEI results suggest changes in precipitation deficits in those areas will be less than in the North Island (Figure 4).

Discussion

This study has provided the first data to show that adult ABB feeding intensity can increase when feeding on drought-stressed plants independently of endophyte infection status. Insect performance is determined by the food ingestion of quality host plants (Wetzel et al. 2016). Insects require proteins, amino acids, carbohydrates, fatty acids, vitamins and minerals for development, growth, maintenance, and reproduction (Bala et al. 2018). The host plants' chemical composition, and thus host plant quality, can be influenced by environmental conditions, such as drought (Lin et al. 2022), which affects the fitness and growth of herbivorous insects (Kuczyk et al. 2021; Hewitt et al. 2023). One explanation for increased feeding in drought-exposed plants is the higher nitrogen and crude protein levels indicated on the negative direction of PC1 "drought axis". Nitrogen is a key nutrient for insect development since it increases biosynthesis and the accumulation of proteins, sugars, and amino acids (Bala et al. 2018). There is a positive relationship between the host plant's nitrogen concentration and insect performance (Kurze et al. 2018; Raharivololoniaina et al. 2021). A comparable increase in foliar nitrogen was measured in drought-stressed brassica plants (Valim et al. 2016). Plants require nitrogen to support cellular changes to maintain function, but this is limited by their ability to access soil nitrogen when soil moisture levels are low. Plants can resorb nitrogen from senescing tissue and internal plant N stores to maintain plant function (Yuan and Li 2007).

Another aspect to consider in explaining the increased diet consumption of drought-exposed plants is a change in the foliar carbon: nitrogen (C:N) ratio. The PCA shows that drought may have reduced the C:N ratio, thereby diminishing the nutritional quality of the plant tissue by reducing the concentration of amino acids and proteins (N), in line with other findings (Docherty et al. 1997; Hamann et al. 2021). To compensate, herbivorous insects increase their feeding (Stiling and Cornelissen 2007). A meta-analysis comprising 79 studies reported that foliar C:N ratios generally decreased under drought (Sun et al. 2020). Thus, nitrogen availability alone might not predict the response of insect herbivores to drought-induced changes in plants (Hale et al. 2003; Mody et al. 2009; Banfield-Zanin and Leather 2015). Field studies showed that the abundance of bird cherry-oat aphid (*Rhopalosiphum padi* L.) was reduced by

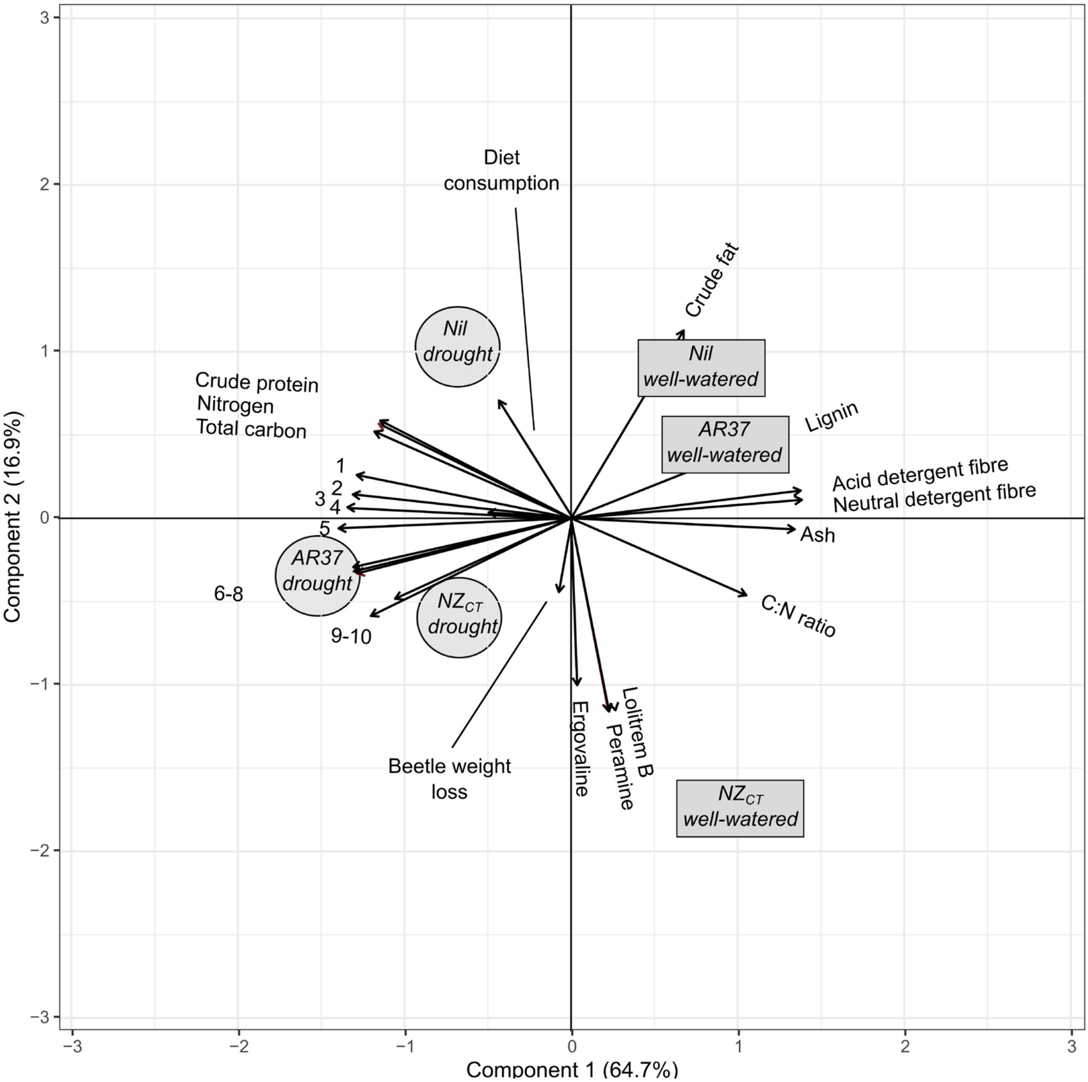

Figure 3 Biplot of a principal component analysis (PCA) of alkaloids (mg/kg), plant hormones (mg/kg), and nutritional value of plant material (%DM) in drought-exposed (circles) and well-watered (rectangles) perennial ryegrass plants infected with *Epichloë* (AR37, NZ_{CT}) and *Epichloë*-free plants (Nil). Vectors with numbers indicate 1 = indole-3-acetic acid, 2 = salicylic acid, 3 = total epoxyjanthitrems, 4 = organic matter, 5 = abscisic acid, 6 = soluble sugars, 7 = digestibility of organic matter, 8 = metabolisable energy, 9 = organic matter digestibility in-vivo, 10 = non-structural-carbohydrates.

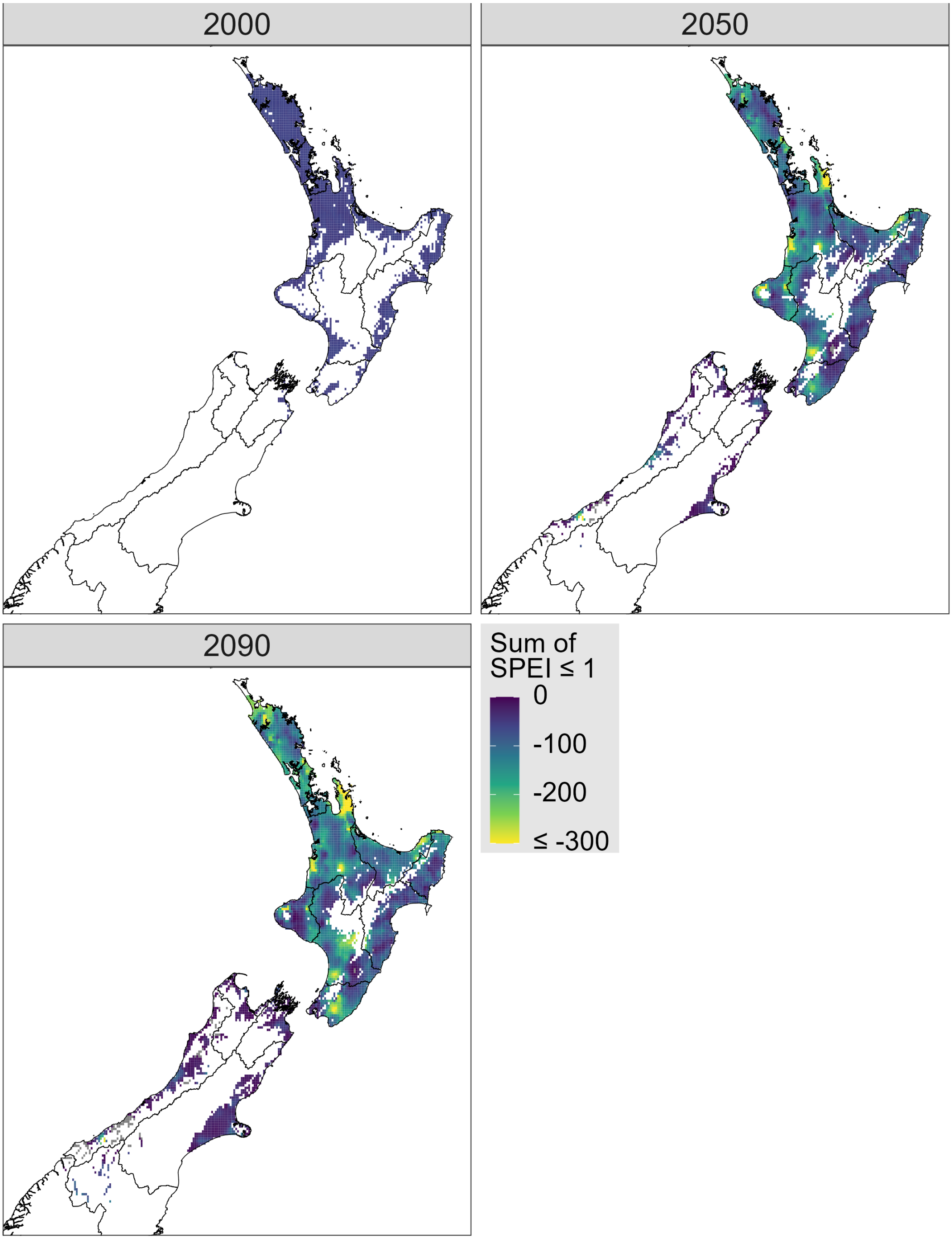

Figure 4 New Zealand locations predicted to be warm enough for African black beetle in 1991-2010 (2000), 2041-2060 (2050) and 2080-2099 (2090) (all shaded areas). Variation in depth of shading (see legend) in 2050 and 2090 shows estimates of potential drought severity based on the Standardised Precipitation Evapotranspiration Index (SPEI) using 2000 as the reference period. Fine black lines indicate Regional Council boundaries.

50% in drought-stressed wheat in comparison to well-watered plants, despite the increased nitrogen availability in the drought-exposed plants (Johnson et al. 2011). Therefore, insects' response to drought may differ based on the feeding guild (Huberty and Denno 2004), taxonomy, and the intensity of water stress (Tariq et al. 2012; Sconiers et al. 2020; Kansman et al. 2022). Plants have evolved many inducible traits that directly (e.g., yield and root development) and indirectly (e.g., osmotic adjustment and regulation of plant hormones) protect against water availability fluctuations (Zhang et al. 2012; Waadt et al. 2022). For example, the levels of IAA, ABA (abscisic acid) and SA (salicylic acid), three endogenous plant stress signalling hormones, increase under drought stress (Krishnan and Merewitz 2015), controlling stomatal aperture and osmotic adjustment (Srivastava 2002; Torun 2019). Our results corroborate this, with higher IAA, ABA, and SA levels in drought-exposed plants (Figure 3). Phytohormone defence against drought may also increase resistance to insect herbivores (Nguyen et al. 2016; Blundell et al. 2020; Lin et al. 2021).

A further important aspect of the differences between feeding intensity of ABB on plants is the presence of fungal endophytes. Fungal alkaloids were key to reducing ABB diet consumption in the bioassay as indicated by the inverse relationship of diet consumption and alkaloids ergovaline, lolitrem B, and peramine (Figure 3). Our results showed that adult ABB fed most on diets containing endophyte-free drought-exposed plants. In a drought scenario, plants infected with NZ_{CT} and AR37 reduced diet consumption in comparison to endophyte-free plants (Figure 1). AR37 has a strong effect on adult ABB reducing plant damage (Ball et al. 1994) and beetle populations in the field by reducing oviposition (Hume et al. 2007; Thom et al. 2013). Ergopeptines (e.g., ergovaline) produced by NZ_{CT}, have been previously shown to deter adult ABB, at concentrations as low as 1 mg/kg (Ball et al. 1997). Similar ergovaline levels found in the present study are in line with previous research observing higher ergovaline levels in response to water stress (Hahn et al. 2008). Most of the fungal alkaloids were also correlated with beetle weight loss, as indicated by the negative directional value on the PC2. An exception were the epoxyjanthirems, that were found on the negative "drought-axis" on the PC1. This can be partly explained because epoxyjanthirem concentrations were higher in droughted material in comparison to well-watered (Table 2). However, another reason that the epoxyjanthitrems behave differently on the PCA biplot is a product of the experiment itself. Rather than differences in alkaloid spectra occurring along a gradient, each of the three grass treatments have unique alkaloid signatures. As epoxyjanthirems are not produced by NZ_{CT}, and as lolitrem B, peramine and ergovaline are not produced by AR37-infected associations, we would expect the vectors for the two groups of alkaloids to point in different directions in the PCA. As the NZ_{CT} association produces three alkaloids and the AR37 association only contributes one alkaloid, it is not surprising that the greatest contributor to PC2 is the NZ_{CT} association. However, we would also not expect the epoxyjanthitrems vector to point strongly towards the Nil endophyte treatment, as Nil plants do not have epoxyjanthitrems. Consequently, it is roughly perpendicular to PC2 and points towards the drought side of the plot. Thus, although epoxyjanthirems are separated from other fungal alkaloids in the PCA, they still likely contribute to adult ABB deterrence (A. Popay, pers. communication). ABB generally lost weight during the 2-week bioassay which is likely attributable to beetle age. ABB were collected in spring, a time when overwintered adults emerge and mate, and were approaching the end of their natural lifecycle.

Risk to New Zealand pastures

Poor persistence is one of the most important factors limiting pasture performance (Smith and Brazendale 2011). Drought and insect pest pressure are the biggest drivers of pasture decline (Clark 2011; Zydenbos et al. 2011; Hewitt et al. 2021; Hewitt et al. 2023). Our predictions of ABB's potential distribution in 2000 and 2050 were similar to those obtained by Mansfield et al. (2021) who used different climate data, with the most noticeable difference occurring in 2050 when we predicted less of the South Island being warm enough for ABB. The expectation that drought severity will increase with time in many areas that are, or will become, warm enough for ABB to persist was also consistent with previous analyses (Sood and Mullan 2020; Vicente-Serrano et al. 2022). Much of this increased severity will be due to greater evapotranspiration in warmer temperatures rather than to deficits in precipitation alone (Sood and Mullan 2020; Vicente-Serrano et al. 2022). In the face of climate change, fungal *Epichloë* endophytes are likely to become an increasingly important constituent of managed pastoral systems for mitigating the effects of concurrent biotic and abiotic stressors. As ABB's distribution continues to increase in New Zealand with climate warming, endophytes will become increasingly important for managing ABB. Further field trials are necessary to improve our understanding of how pasture-insect interactions will be affected by the impacts of climate change.

Conclusions

Understanding the effect of water stress on insect herbivory is critical for predicting and managing outbreaks of agricultural pests in a changing climate.

Fungal alkaloids deter ABB, but as plants become water-stressed their nutritional status may change, potentially increasing their attractiveness for feeding and thereby reducing the efficacy of deterrents and plant defence hormones. Our results suggest that interactions between drought, plant-herbivore relationships and temperature could increase ABB's impacts as New Zealand's climate changes, and areas such as inland Taranaki, Manawatū-Whanganui and Wairarapa could become increasingly at risk.

This experiment has illustrated the importance of *Epichloë* endophytes in managed pasture systems under simultaneous biotic and abiotic stress, a combination often seen in natural settings. Field trials are necessary to determine whether this effect is seen *in situ*, where numerous additional factors will be operating at the same time.

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