

Prospects of *Epichloë* endophytes on fall armyworm growth and survival in tall fescue

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Abstract

Fall armyworm (*Spodoptera frugiperda*) larvae are detrimental pests to many crops in the Southeastern USA, including tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.), grown for turf and forage. In most natural and managed ecosystems, tall fescue plants harbour an *Epichloë* endophyte that imparts protection to the grass from abiotic and biotic stresses, including insect deterrence. Enhancing tall fescue resistance to fall armyworms may be achieved by identifying *Epichloë* endophyte strains that exhibit antibiosis or antixenosis. In this study, we inoculated 22 *Epichloë* strains (16 non-toxic and 6 toxic), isolated from tall fescue collections of various origins, into an endophyte-free continental-type tall fescue cultivar and a Mediterranean-type tall fescue cultivar. Leaves from each strain × host combination were used in feeding studies with fall armyworm larvae. We investigated the effect of different strain × host combinations on fall armyworm development by measuring larval weight gain, days to pupation, and survival in the laboratory. Complementary ratings of defoliation were conducted in a field infested with fall armyworm to assess pest damage under natural conditions. The Mediterranean-type tall fescue, in combination with most strains, showed detrimental effects on larval development. Two endophyte strains (one capable of producing mammalian-toxic ergot alkaloids and the other not capable of producing ergot alkaloids) significantly reduced fall armyworm larval development and minimised defoliation in the field. These will be characterised further for use in integrated pest management.

Keywords: Biological control, *Festuca arundinacea*, forages, insect resistance

Introduction

Tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.) is a prevalent, cool-season perennial grass grown on about 15 million hectares in the USA (Young et al. 2014). It is economically significant worldwide for forage and turf production. Three morphotypes of tall fescue exist: continental, Mediterranean, and rhizomatous (Hand et al. 2010). Continental types dominate forage production in temperate climates, while Mediterranean types are adapted to dry, hot summers. Morphologically, continental types have broader, thicker leaves compared to the softer, narrower blades found in Mediterranean types. Rhizomatous tall fescue is primarily utilised for turf grass.

In most natural and managed ecosystems, tall fescue plants harbour a fungal endophyte, *Epichloë coenophiala* (Morgan-Jones & W.Gams) C.W.Bacon & Schardl (formerly *Neotyphodium coenophialum*), which imparts improved abiotic and biotic stress tolerance, including protection against insects, phytopathogens, and drought (Lee et al. 2021). These endophytes produce various classes of alkaloids such as peramines, lolines, indole-diterpenes, and ergot alkaloids (Young et al., 2014). Peramine and lolines are crucial for the anti-insect bioactivity exhibited by tall fescue plants. While protective to the plant, ergot alkaloids cause a detrimental condition to livestock called fescue toxicosis (Hill et al. 1994), prompting research to identify endophyte strains that are safe for grazing mammals while exhibiting bioactivity towards invertebrate pests (Bouton, 2009).

Fall armyworm (*Spodoptera frugiperda*) J.E.Smith is a major pest in the USA and worldwide, capable of producing multiple generations per year in warm climates. In northern regions of the USA, it cannot

survive over winter but re-colonises annually via migration from overwintering populations from southern states. In the Southeastern USA, it can persist and feed year-round (Capinera, 1999; Johnson, S.J. 1985; Sparks, 1979). The larval stage is responsible for crop damage, feeding on over 350 plant species, with primary hosts including maize, sorghum, rice, and cotton, as well as turf and forage grasses in the USA (Montezano et al. 2018). Prior studies on turf perennial ryegrass (*Lolium perenne* L.) and various *Festuca* spp. showed that *Epichloë* endophyte-mediated resistance to fall armyworms was variable among and within species of grasses and endophytes (Breen, 1993; Hardy et al. 1985). However, only limited research has focused on tall fescue-endophyte interactions for fall armyworm control in forage production. Chemical studies implicate alkaloids as deterrents to insect pests (Clay & Cheplick, 1989; Keathley & Potter, 2012), and there is increasing interest in the USA for exploiting *Epichloë* strains for insect resistance (Kuldau & Bacon, 2008; Lee et al. 2021).

This study evaluates the effect of 22 *Epichloë* strains, including livestock toxic and livestock non-toxic types, inoculated into a continental and a Mediterranean tall fescue background, to identify promising combinations for fall armyworm deterrence.

Materials and Methods

Plant material preparation

In 2015, 1,075 tall fescue accessions from diverse regions worldwide were planted at the University of Georgia's J. Phil Campbell Research Center in Watkinsville, GA, USA (33.8834°N, 83.4304°W). After screening for endophyte presence and ergot alkaloids using the Phytoscreen Ergot Alkaloid Kit (Cat #ENDO899-2t, Agrinostics Ltd., USA), 23 accessions with ergot alkaloid-free endophytes and five ergot alkaloid-containing accessions were selected. Additionally, plants infected with the commercial livestock safe endophytes MaxQ and MaxQII were planted in the field.

Endophyte strains were isolated from dissected grass tillers. Tillers were surface disinfected (95% ethanol, 75% ethanol, 50% bleach) and cultured on potato dextrose agar (PDA) at 22°C in the dark for a minimum of one month. Cultures were maintained in these conditions for up to 6 months. Endophyte-free seeds of the Mediterranean-type tall fescue cultivar cv. Prosper and a continental-type experimental population GALA 1402 were surface disinfected with bleach and germinated on 3% water agar and incubated at 22°C in the dark for approximately a week, until young seedlings emerged. A small amount of endophyte culture was

inoculated into the meristematic region of the newly germinated seedlings with the aid of a dissecting microscope. Prior to inoculation, endophyte cultures were prepared by grinding a piece of endophyte culture with a sterile mortar and pestle with potato dextrose broth, pouring the ground culture broth mixture into a Petri dish, and gently shaking in a dark incubator at 22°C for 1 week. Following inoculation, the seedlings were incubated in a growth chamber, then transferred to soil, and assessed for endophyte infection using seedling kits (Cat #ENDO797-2, Agrinostics Ltd., USA). Successfully infected plants were transplanted in the field for seed bulking. Eventually, 20 endophyte-host combinations were obtained for GALA 1402, and 16 in Prosper (Table 1).

Feeding trials

Fall armyworm larvae (second instar) were obtained from a laboratory colony (Benzon Research Inc., USA). The larvae were reared on their shipping media for three days before being assigned to treatments at the second instar.

Due to growth chamber space limitations, feeding experiments were conducted in three trials, with new larvae shipped fresh for each trial. Each larva was weighed individually on a high-resolution scale (to 0.0000mg) and placed in a 3.5-inch-diameter plastic cup with 1g of assigned plant material in the form of 1-inch-long fresh cuttings, with one larva per cup. Plant material was collected weekly and stored at 4°C. The plastic cups were covered with plastic wrap and a rubber band used to secure the wrap. Plant material was replaced at every weigh session. Trial 1 used 20 larvae per treatment (20 replications). Trials 2 and 3 used 10 larvae per treatment (10 replications). Endophyte-free (E-) versions of each cultivar served as controls. The cups were maintained at 26°C under a 12-hour light cycle. Trials used plant material grown primarily in the greenhouse, where the material for each treatment came from multiple individual plants for the assigned genotype/endophyte combination.

Measurements included initial and final larval weights measured every other day, survival to pupation, and days to pupation, checked daily. In trial 1, due to low survival, weight gain was measured from initial weight to Day 7; in trials 2 and 3, it was measured from initial to pre-pupation weight. Pre-pupation weight was assessed before the first measurement of weight loss, indicating pupation, within a reasonable timeline.

Field validation of fall armyworm defoliation

To assess natural fall armyworm damage under field conditions, visual defoliation ratings were assessed

Table 1 The strain/host combinations tested in each trial. (T) indicates an endophyte strain that has been identified as toxic based on ELISA testing. (N) indicates an endophyte strain that has been identified as non-toxic to mammals.

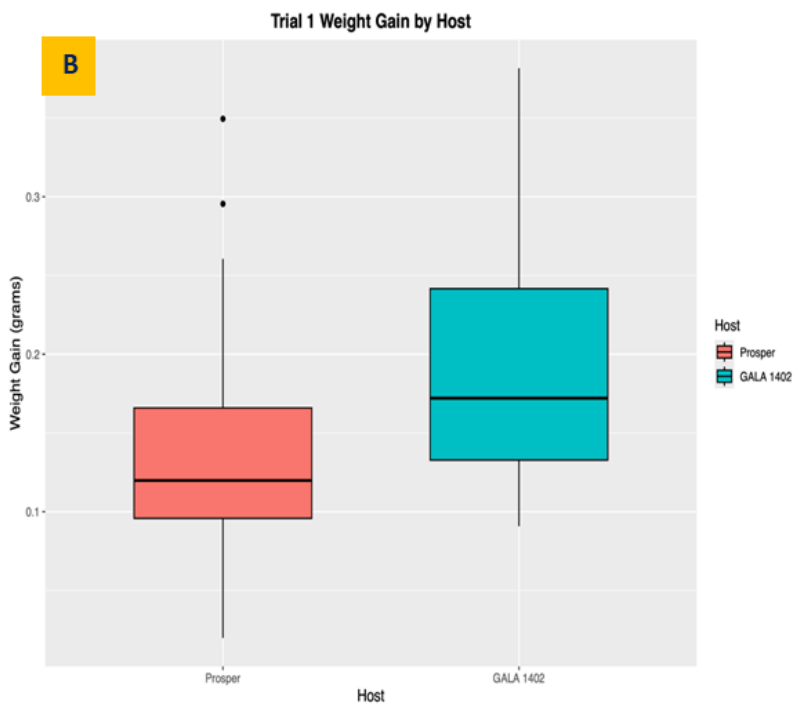
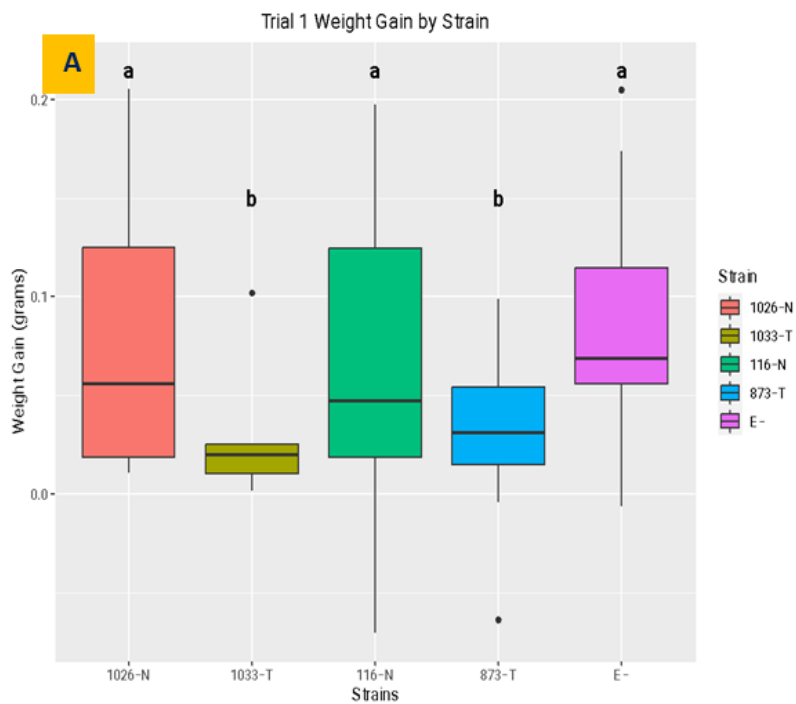
Trial 1	Trial 2	Trial 3
Prosper 116-N	GALA 1402 474-N	GALA 1402 1068-N
GALA 1402 116-N	Prosper 474-N	Prosper 1021-N
Prosper 1026-N	GALA 1402 754-N	GALA 1402 1102-584-N
GALA 1402 1026-N	Prosper 754-N	GALA 1402 106-N
Prosper 873-T	GALA 1402 212-N	Prosper 1028-N
GALA 1402 873-T	Prosper 212-N	GALA 1402 1102-542-N
Prosper 1033-T	GALA 1402 157-N	GALA 1402 1034-N
GALA 1402 1033-T	Prosper 157-N	GALA 1102 MaxQ-N
	GALA 1402 1010-N	GALA 1402 1022-N
	Prosper 1010-N	Prosper 1102-584-N
	GALA 1402 939-N	Prosper E-
	Prosper 939-N	GALA 1402 E-
	GALA 1402 217-T	
	Prosper 217-T	
	GALA 1402 1070-T	
	Prosper 1070-T	
	GALA 1402 733-T	
	Prosper 733-T	

on 8 August 2024 at the J. Phil Campbell Research and Education Center in Watkinsville, GA, USA (33.8834°N, 83.4304°W). The field site consisted of blocks of tall fescue endophyte strain combinations established in the fall of 2021. Each plot contained 16-24 plants of the same unique genotype × endophyte strain combinations (Table 1). Each plot ranged in size from 4 × 4 to 4 × 6 plants (16-24 plants per plot), with plants spaced approximately 0.5 metres apart, corresponding to plot dimensions ranging from ~3.5 to 5.5 m². The fall armyworm infestation occurred as a result of a natural outbreak. Defoliation was visually estimated at the plot level by a single observer using a 0-10 scale, where 0 indicated no visible damage and 10 indicated complete defoliation across the plot. These scores were then multiplied by 10 to convert to a standardised 0-100% scale for analysis. At the time of assessment, the region was experiencing the third fall armyworm generation typical for northeastern Georgia, with feeding signs consistent with mid-to-late larval instars. While some cumulative damage from earlier generations may have occurred, the timing of visual assessments primarily captured peak defoliation from

the dominant cohort active in August.

Statistical analysis

Each larval feeding trial was analysed separately. Linear models or generalised linear models, where appropriate, were fitted for each response variable (weight gain, days to pupation, survival) using the *stats* package in R v.4.2.2 (Rstudio, 2022). Residuals were visually inspected via histograms (*graphics* package) and tested for normality via the Shapiro-Wilk test. Two-way ANOVAs were conducted to assess the effects of host genotype and endophyte strain. Significance was declared at $P < 0.05$. *Post hoc* multiple comparisons were performed using the least significant difference (LSD) test at $\alpha = 0.05$ (*agricolae* package v1.3-5). Compact letter displays were generated to denote the statistical significance of groupings. Mean percentage defoliation for field data was calculated per plot (1-2 plots per strain, 16-24 plants per plot). Due to non-normal residuals (Shapiro-Wilk $P < 2.2 \times 10^{-16}$). Generalised linear models (GLMs) with a Gaussian distribution were fitted. Estimated marginal means and Sidak-adjusted pairwise comparisons were calculated



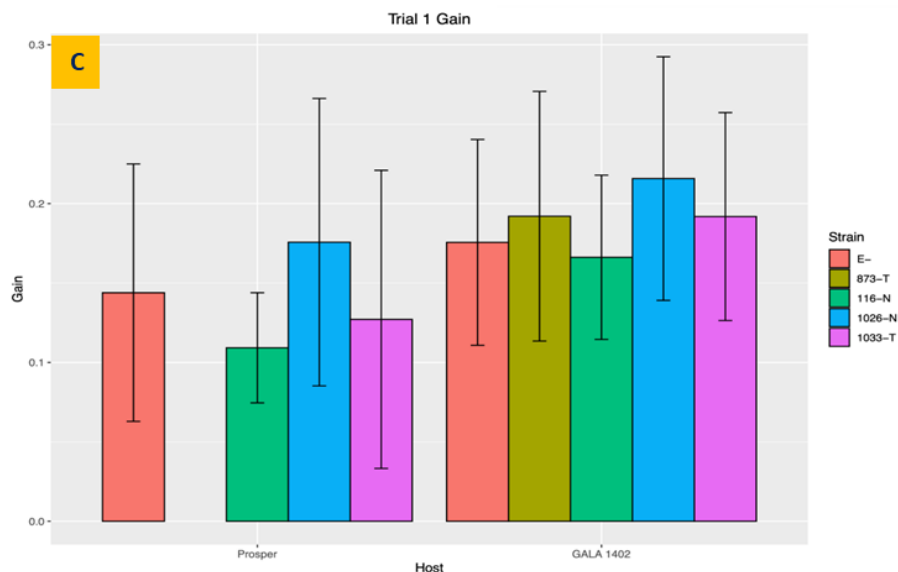


Figure 1 Boxplot of *S. frugiperda* fall armyworm larval weight gain from 25 October to 1 November 2021 by A) *Epichloë* endophyte strain and B) tall fescue host cultivar for trial, and C) *Epichloë* endophyte strain and host. Letters above the bars indicate significance groups determined by LSD at $\alpha=0.05$.

using the ‘emmeans’ package in R v4.2.3. Graphical summaries were created in ggplot2 v4.3.1. (Wickham, 2016).

Results

Larval development in feeding trials

Fall armyworm larval development was significantly affected by host genotype and endophyte strain across all three feeding trials.

Trial 1

Larval weight gain was significantly influenced by both host genotype and endophyte strain ($P<0.001$) (Figure 1), with a significant host \times strain interaction ($P<0.05$) (Figure 1C). Larvae feeding on cv. Prosper E- exhibited significantly lower weight gain compared to those feeding on GALA 1402 E- ($P<0.05$) (Figure 1B). Among endophyte strains, 1033-T and 873-T were associated with the lowest larval weight gains ($P<0.05$) (Figure 1A). Endophyte infection did not seem to impact larval weight gain, with 1033-T and 873-T having comparable results to the E- replicates (Figure 1). These strains are known to produce mammalian-toxic ergot alkaloids but may not produce insect-detering compounds.

Host genotype and endophyte strain also significantly influenced days to pupation ($P<0.001$), with larvae feeding on cv. Prosper exhibiting longer development times than those feeding on GALA 1402 ($P<0.05$)

(Figure 2A, 2B). Larvae feeding on strain 1033-T exhibited the longest mean time to pupation ($18.7 \pm$ SE days; $P<0.05$).

Host genotype was the only significant factor influencing survival ($P<0.01$), with larvae feeding on cv. Prosper suffering higher mortality (70%) than those feeding on cv. GALA 1402 (41%) ($P<0.05$) (Figure 3). No larvae survived to pupation in Prosper with 873-T.

Trial 2

Both host genotype and endophyte strain significantly affected larval weight gain ($P<0.001$ and $P<0.01$, respectively) (Figure 4). Larvae feeding on strain 754-N exhibited the lowest weight gains, followed by strain 212-N ($P<0.05$) (Figure 4A). Larvae feeding on cv. Prosper had lower weight gains compared to GALA 1402 ($P<0.05$) (Figure 4B).

Days to pupation were significantly influenced by both host genotype and strain ($P<0.001$) (Figure 5). Larvae feeding on strains 754-N and 212-N exhibited longer developmental periods compared to other strains ($P<0.05$) (Figure 5A). Larvae feeding on cv. Prosper also required more days to pupation than those feeding on GALA 1402 ($P<0.05$) (Figure 5B). There was a significant interaction between host and strain ($P<0.001$). No significant differences in larval survival were observed among treatments in trial 2 (data not shown).

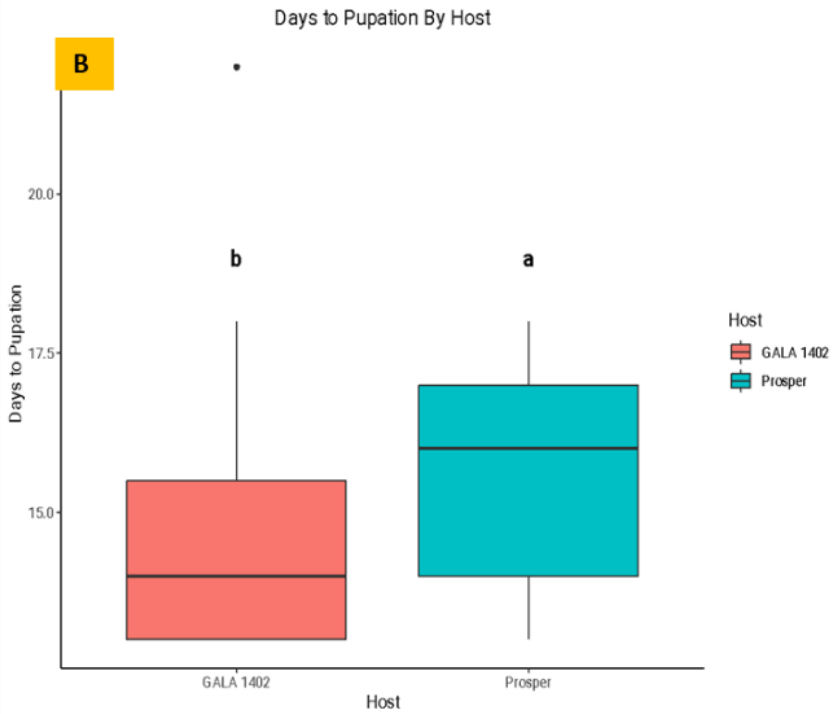
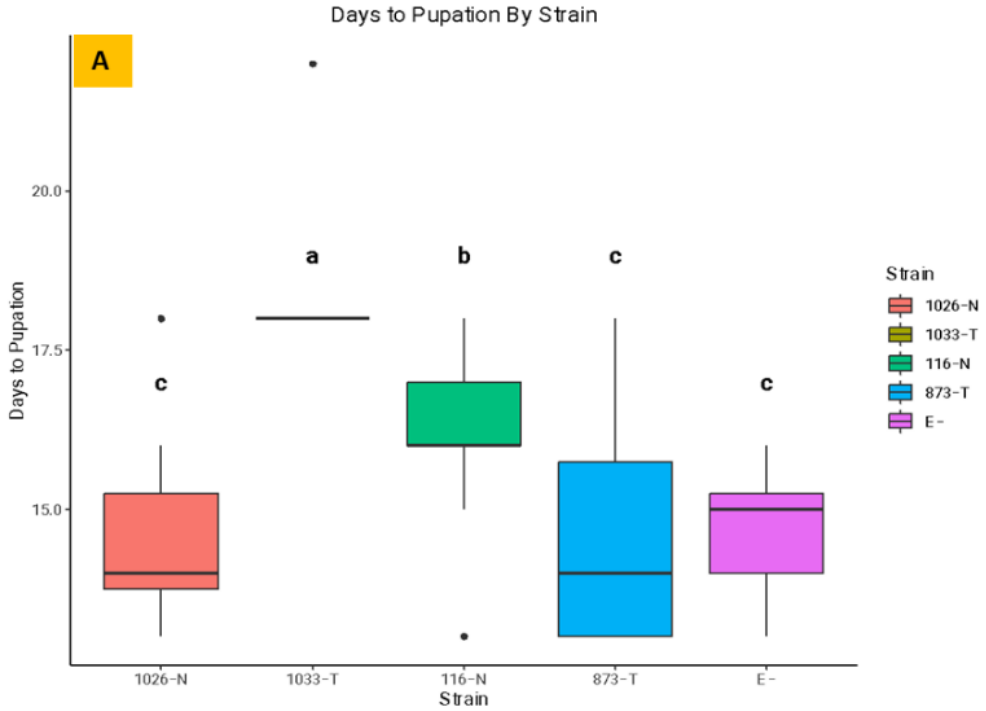


Figure 2 Boxplot of *S. frugiperda* fall armyworm larval days to pupation by A) *Epichloë* endophyte strain, and B) tall fescue host cultivar for trial. Letters above the bars indicate significance groups determined by LSD where $\alpha=0.05$.

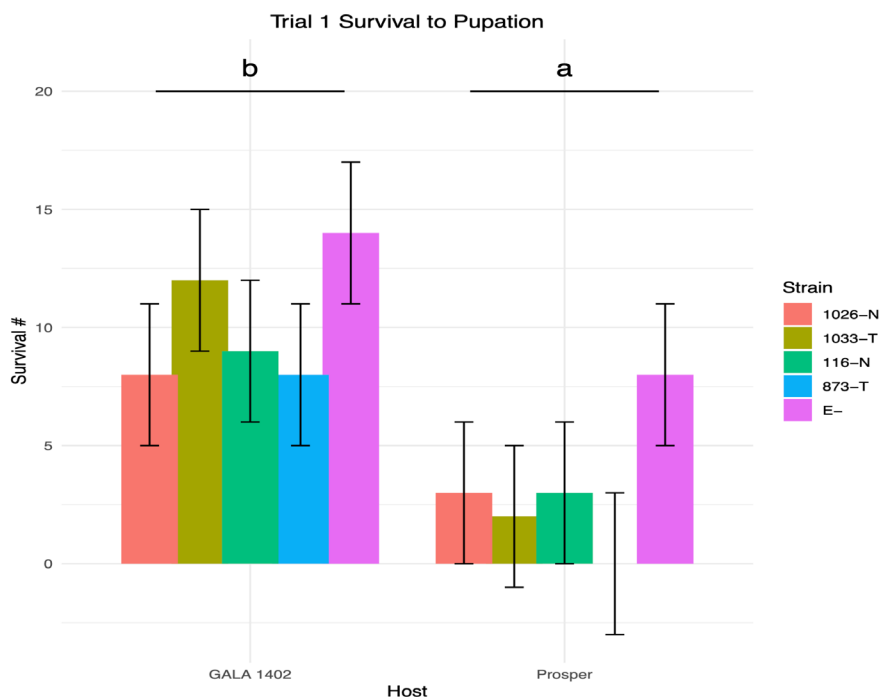


Figure 3 Bar chart of survival to pupation (number alive out of 20 tested per treatment) for fall armyworm larvae by tall fescue host cultivar and *Epichloë* strain for trial one. Letters indicate significance groups between hosts, determined by LSD where $\alpha=0.05$, and error bars indicate standard error.

Trial 3

Larval weight gain was significantly affected by endophyte strain ($P<0.05$) but not by host genotype (Figure 6). Strain 1021-N resulted in the lowest larval weight gains (mean 0.15 g), significantly lower than all other strains ($P<0.05$) (Figure 6).

Days to pupation were significantly affected by both strain ($P<0.001$) and host genotype ($P<0.05$) (Figure 7). Larvae feeding on strain 1021-N exhibited the longest developmental periods ($P<0.05$) (Figure 7A). Larvae feeding on cv. Prosper required more days to pupation compared to cv. GALA 1402 ($P<0.05$) (Figure 7B). No significant differences in larval survival were detected in trial 3 (data not shown).

Field defoliation rating

Mean percent defoliation per plot across tall fescue plots containing the same genotype x endophyte strain combinations used in the feeding assays varied in defoliation levels by strain (Figure 8). Average defoliation ranged from 20% to 90%. Fall armyworm caused the highest levels of defoliation to plots with strain 116-N (90%), whereas strains 1021-N and 212-N were defoliated the least (20%) (Figure 8).

Among the five strains that showed the lowest feeding damage fall in the laboratory feeding assays

(873-T, 754-N, 1033-T, 1021-N, and 212-N), in the field assessment, strains 873-T, 1021-N and 212-N showed the lowest average field defoliation, while strain 116-N showed the highest. There were no E- plots located in the area of the field where the fall armyworm outbreak occurred, so E- plots were not assessed for either genotype.

Discussion

This study demonstrated that *Epichloë* endophyte strains can differ significantly in their ability to interfere with fall armyworm herbivory of tall fescue. Larval development (weight gain, time to pupation), along with intensity level of defoliation under field conditions of plants varied with both endophyte strain and host genotype, supporting the hypothesis that specific endophyte-host combinations can influence pest resistance and tolerance. Notably, proprietary strains 212-N and 1021-N consistently reduced both larval performance in the laboratory and field defoliation, suggesting strong anti-herbivory effects. As neither of these strains are yet available in commercial tall fescue and outperformed current commercially available strains, they are strong candidates for further associative breeding work. We did not find that ergot alkaloid containing endophytes had stronger antibiotic

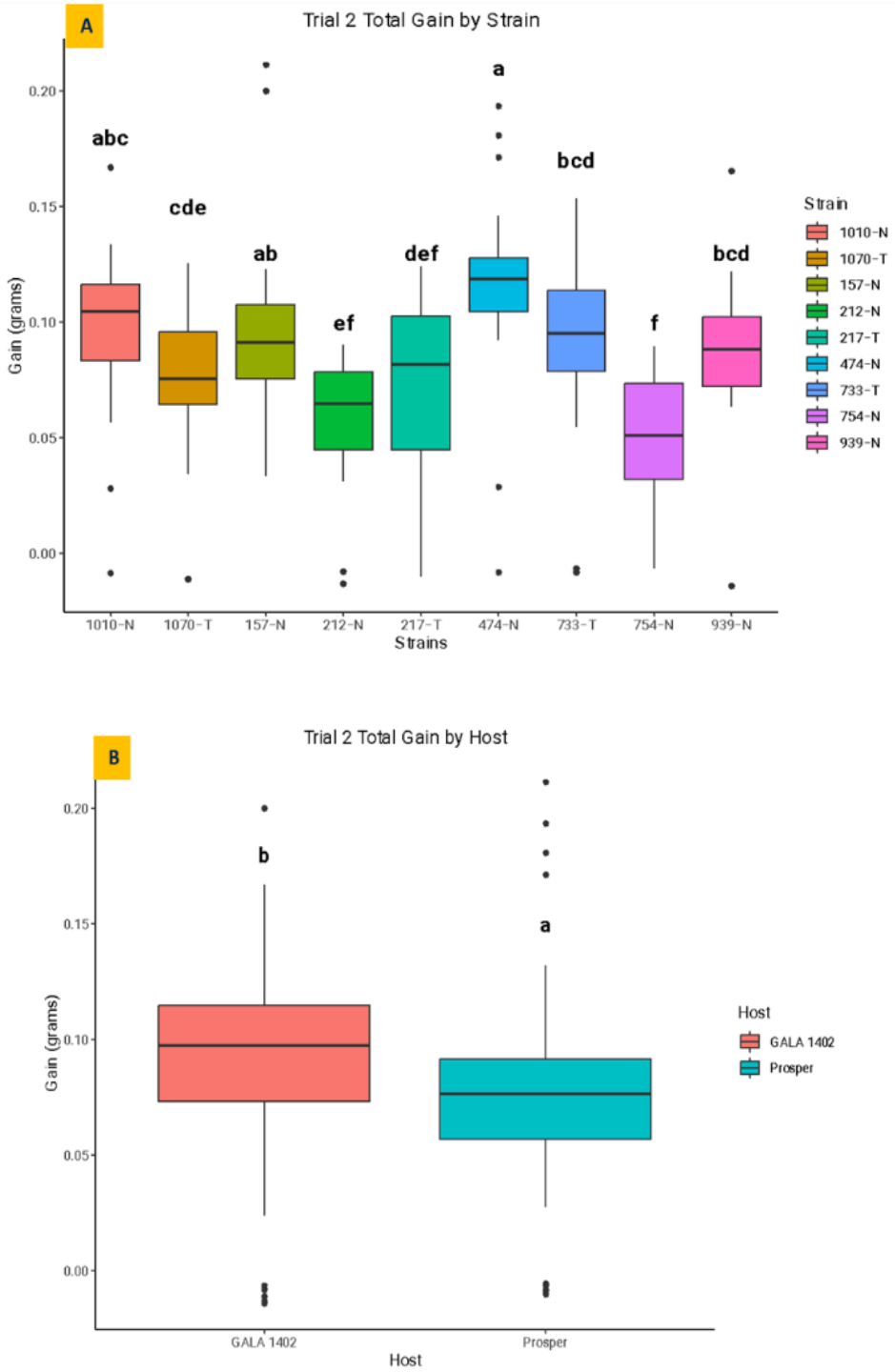


Figure 4 A boxplot showing total weight gain (Y axis) of fall armyworm larvae by A) *Epichloë* strain and B) tall fescue host cultivar for trial 2. Letters indicate significance groups determined by LSD with $\alpha=0.05$.

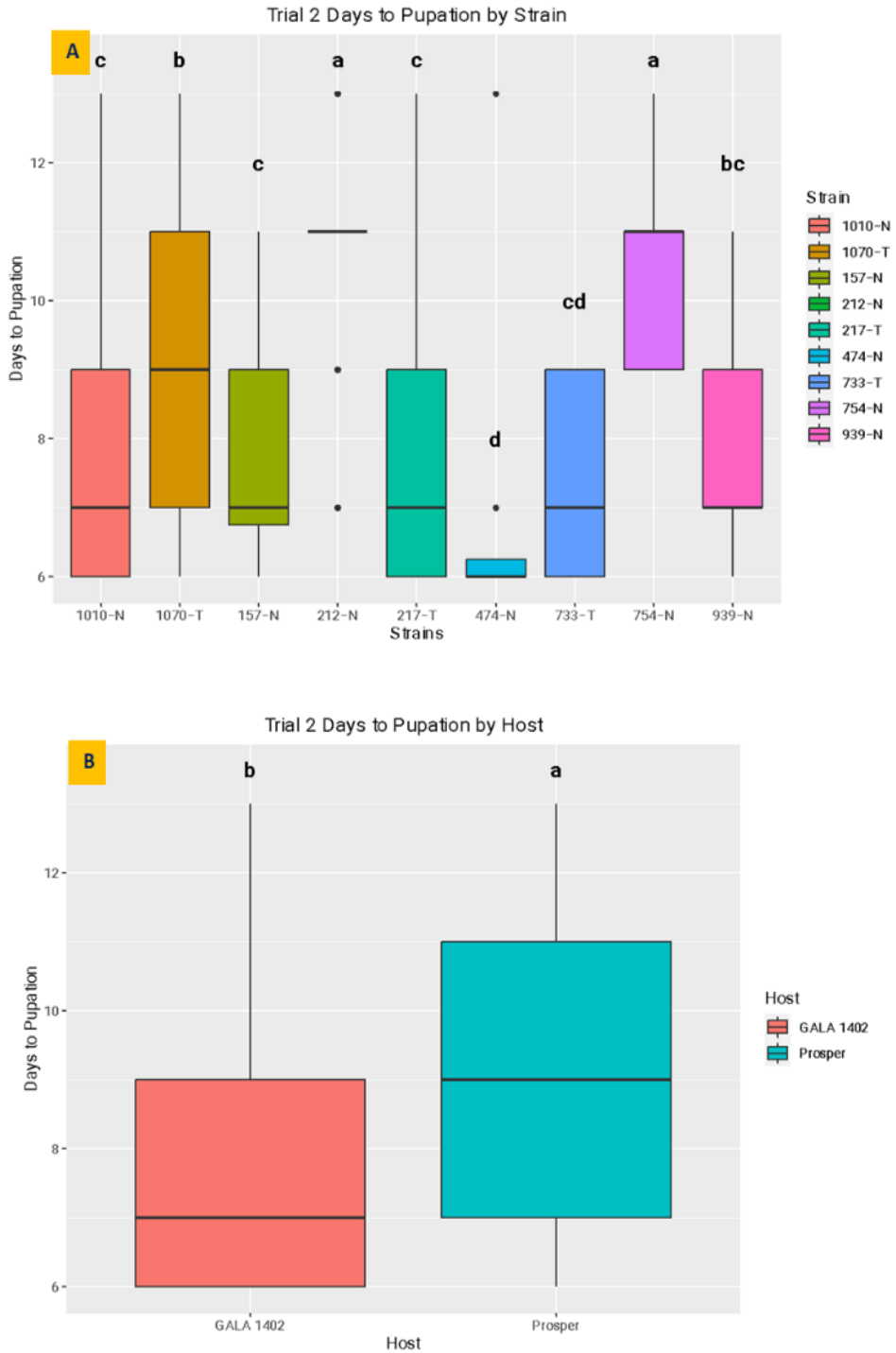


Figure 5 Boxplot of days to pupation (Y axis) for fall armyworm by A) endophyte strain and B) host for trial 2, letters indicate significance groups determined by LSD where $\alpha=0.05$.



Figure 6 Boxplot of larval fall armyworm weight gain (Y axis) by endophyte strain for trial 3. Letters indicate significance groups determined by LSD with $\alpha=0.05$.

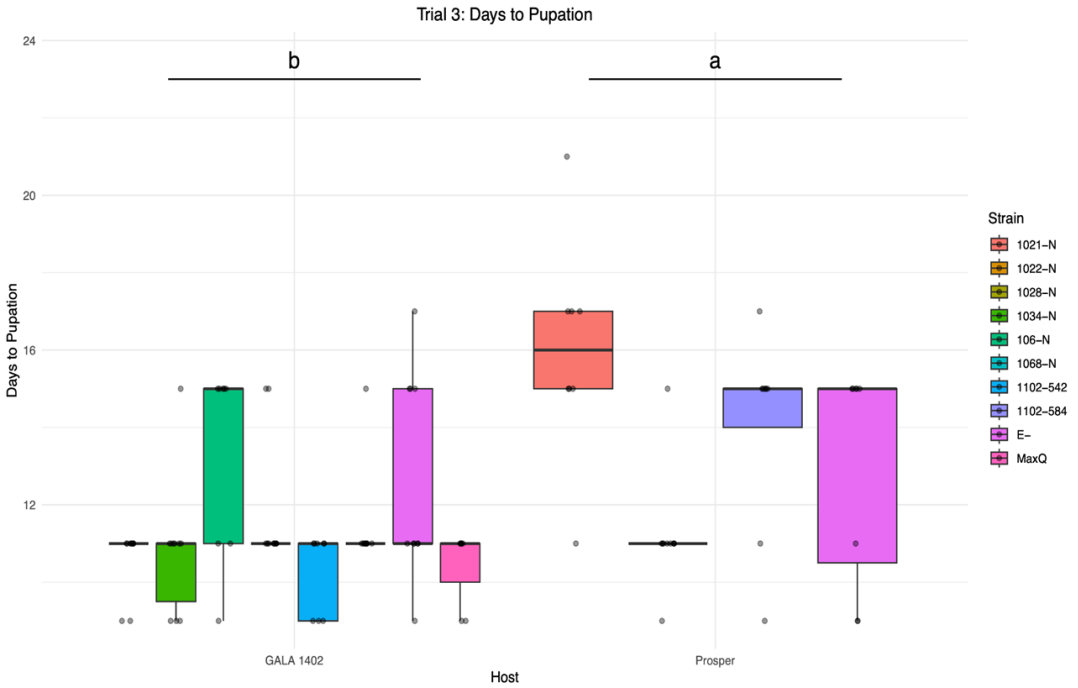


Figure 7 Boxplot of larvae days to pupation (Y axis) by endophyte strain and host for trial three, letters indicate significance groups determined by LSD where $\alpha=0.05$.

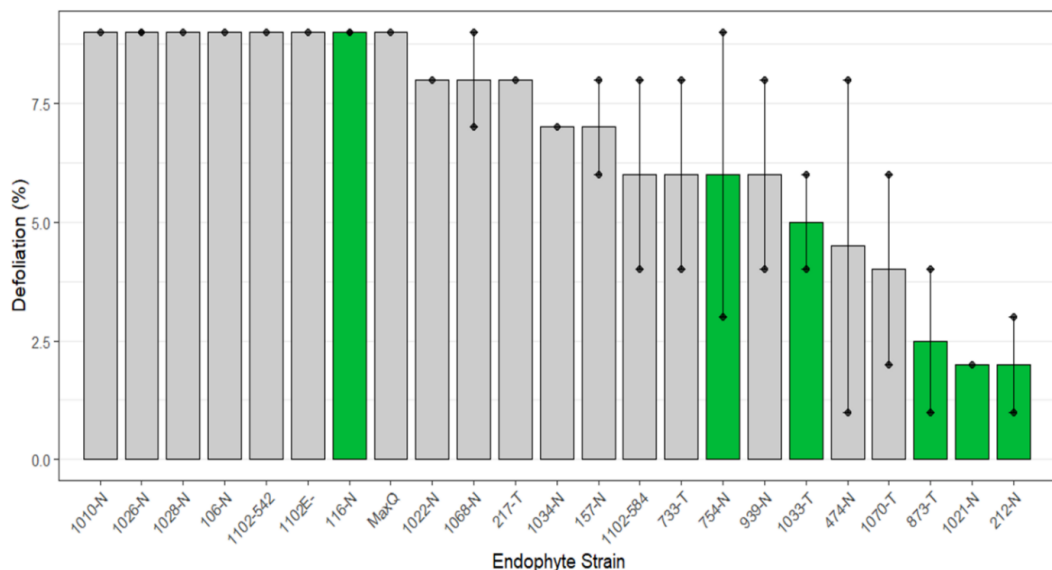


Figure 8 Estimated percent mean defoliation (\pm SE) per plot (%) in 1-2 plots containing 16-24 tall fescue plants inoculated with different *Epichloë* strains. Strains highlighted in green were found to significantly impact larval growth and/or development in the separate laboratory feeding assays.

associative breeding work. We did not find that ergot alkaloid containing endophytes had stronger antibiotic effects than ergot alkaloid-free endophytes. Our findings align with previous work on endophyte-mediated insect resistance in fescue and ryegrasses (Breen, 1993; Hardy et al. 1985), which indicated that certain endophyte strains reduce herbivore growth and survival. However, earlier studies focused largely on perennial ryegrass or a limited set of tall fescue strains. By examining a broader diversity of *Epichloë* strains across two host genotypes, our results extend current understanding and identify novel strain x host combinations with enhanced biocontrol potential.

Some variability in defoliation was observed among strains in the field, which may reflect biological variation and/or environmental factors. Host genotype also influenced outcomes, highlighting that the effectiveness of endophyte strains in deterring pests may be dependent on their compatibility with specific host plant genetic backgrounds. These differences may be reflective of ecotype-linked physiological differences, as Mediterranean tall fescue has narrower blades relative to broader-leaved continental-type tall fescue, which may impact alkaloid accumulation and forage quality, therefore influencing insect feeding behaviour.

Overall, these results advance our understanding of endophyte-mediated pest resistance in tall fescue

and suggest that careful selection of strain x host combinations can substantially enhance pasture pest tolerance. Future studies will be focused on alkaloid profiling of promising strains, long-term field performance evaluations, and broader testing against additional insect species to fully characterise the basis of deterrence.

Practical implications

This study identifies two novel *Epichloë* strains with the potential to enhance tall fescue resistance to fall armyworms under both controlled and natural outbreak conditions. Incorporating these strains into tall fescue germplasm could reduce reliance on chemical insecticides, promoting sustainable forage production and minimising environmental impacts. These results underscore the value of expanding endophyte strain diversity and highlight the practical importance of screening candidate strains across different genetic backgrounds and multiple insect species. Matching effective endophyte strains to compatible host genotypes will be critical for optimising field performance and pest suppression outcomes.

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