

Volatile Compounds in Endophyte Infected *Festuca arundinacea*: Impact on *Heteronychus Arator* Host Selection

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Abstract

Mediterranean (M) and Continental (C) *Festuca arundinacea* cultivars in Australia are either endophyte-free (E^-) or uniquely infected with the fungal endophyte *Neotyphodium coenophialum* strain AR⁵⁴² (E^{AR542}). The impact of volatile oils produced by Mediterranean and Continental cultivars in response to E^{AR542} on enhancing the grass's resistance to insects is still largely unknown. In this study, we determined the volatile-oil profiles produced by ME^- , CE^- , ME^{AR542} and CE^{AR542} cultivars and their impacts on the host-selection behavior of *H. arator* adults.

A total of 22 volatile compounds were detected in all *F. arundinacea* samples tested irrespective of being from Mediterranean or Continental cultivars and with or without endophyte. Octanal, 2-ethylhexanol, dodecane, (*Z*)-2-hexen-1-ol, butyl hexanoate, (*Z*)-3-hexen-1-ol, 1-octen-3-ol, and butylated hydroxytoluene were the major compounds identified and variably influenced by the germplasm and endophyte infection status. Quantitatively, ME^- expressed lesser quantities of 1,6-octadien-1-ol, hexanal, hexanol, 1-octen-3-ol and ethyl-3-hydroxypentanoate compared with CE^- but greater quantities of butylated hydroxytoluene and hexylhexanoate. Compared with ME^- , the quantities of octanal, 2-ethylhexanol, pentanal, hexanal, undecanal, 1-octen-3-ol, (*Z*)-2-octen-1-ol and clovene were greater in ME^{AR542} . However, compared with CE^- , hexanal, undecanal, undecane, i-octen-3-ol, (*Z*)-2-octen-1-ol, cloven and butylated hydroxytoluene were greater in CE^{AR542} . In four-choice assay, the percentage of the numbers of *H. arator* adult males choosing one of the choices ME^- , ME^{AR542} , CE^- or CE^{AR542} was not significantly different compared to females choosing any of the respective choices. The impact of the germplasm on the host selection of *H. arator* adults was determined using two-choice assay olfactometer. The *H. arator* adults significantly ($\chi^2 = 10$ d.f. = 1, $P < 0.002$) preferred ME^- compared with CE^- . In contrast, the CE^{AR542} was significantly ($\chi^2 = 26.67$, d.f. = 1, $P < 0.05$) preferred host by same beetles compared with ME^{AR542} .

Mediterranean germplasm is different to the Continental germplasm in relation to the quantities of volatile compounds produced and these quantities in Mediterranean and Continental cultivars variably influenced by E^{AR542} infection. Whether changes in quantities of the volatile compounds in E^{AR542} -infected grasses impose any agricultural or ecological impact require further investigation.

Introduction

Summer-active 'Continental' and winter-active 'Mediterranean' are the two commercial germplasms of *Festuca arundinacea* (tall fescue and also known as *Lolium arundinacium*) sown increasingly in Australian pasturelands (Young et al. 2013). Unlike USA, where *F. arundinacea* cultivars are infected with the fungal endophyte *Neotyphodium coenophialum* 'wild type' strain (E^{WT}), in Australian, *F. arundinacea* cultivars sown for grazing systems – either Mediterranean or Continental germplasm – are either endophyte-free (E^-) or infected with the strain E^{AR542} . The presence of E^{WT} in Australian cultivars is extremely rare (Caradus and Johnson 2020; Van Heeswijck and McDonald 1992).

The endophyte *N. coenophialum* either E^{WT} or E^{AR542} forms a unique mutualistic/symbiotic relationship with the host grass, *F. arundinacea*, causing an increase in the yield (Clay and Schardl 2002; Schulz et al. 1999). This increase in the yield is attributed to the increased resistance of the grass to number of abiotic and biotic stress including insects feeding on the host grass (Hewitt et al. 2021; Katrin et al. 2021; Popay et al. 2005; Popay et al. 2009). Adults and larvae of *Heteronychus arator* are a serious threat to the persistence of *F. arundinacea*. Adult beetles generally feed on the stems and roots of *F. arundinacea* either at or just below ground level (King and Watson 1982). The presence of *N. coenophialum* E^{WT} strain is reported to increases the persistence of *F. arundinacea* in areas infested with insects, such as *Aploneura lentisci* (Jensen and Popay 2006), *Balanococcus poae* (Pennell and Ball 1999), *Listronotus bonariensis*, and *H. arator* (Popay et al. 2005).

Although increased *F. arundinacea* persistence was related to number of alkaloids released in endophyte-infected grasses (Caradus and Johnson 2020), other secondary metabolites are reported to play a role including volatile organic compounds (Qawasmeh et al. 2015). Volatile compounds – in addition to alkaloids – are key compounds involved in mediating the grass's resistance to insects (Fuchs and Krauss 2019; Kumar et al. 2021; Qawasmeh et al. 2015). They can act as signals in deterring herbivorous insects or attracting and their natural enemies and can convey complex information regarding plant location, identity, and condition (Hennessy 2020; Pańka et al. 2013; Schulz et al. 1999).

Festuca arundinacea E⁻ produces a mixture of volatile compounds (Tava et al. 1991; Yue et al. 2001). The most-abundant volatile oils detected in *F. arundinacea* infected with *N. coenophialum* E^{WT} are (Z)-3-hexenal, (E)-2-hexenal, hexanol, (Z)-3-hexen-1-ol and (Z)-3-hexen-1-ol acetate, which are commonly known as the green-leaf volatiles (Yue et al. 2001). Other volatile compounds present at lesser quantity are nonanal, decanal, (E, Z)-2,6-nonadienal, and 1-octanol.

Several factors have been reported to influence the levels of volatile compounds in *F. arundinacea* (Mayland et al. 1997). Examples of these factors include the genotype and phenotype characters of the cultivar, and the infection with the endophyte *N. coenophialum* strain E^{WT}. Cultivars from Mediterranean and Continental germplasms of *F. arundinacea* have been reported to produce generally similar levels of volatile compounds (Mayland et al. 1997; Tava et al. 1995). Levels of hexanal, 1-octen-3-ol, nonanal, phenyl acetaldehyde, octanal, and (Z)-3-hexen-1-ol in the Continental cultivars Barcel, Clarine, and Magno were similar and were not significantly different compared with their levels in the Mediterranean cultivars M. Kasba, Sopline, and Tanit (Tava et al. 1995). The volatile (E)-2-hexenal was the only compound detected at statistically significant higher levels in the Mediterranean cultivars M. Kasba and Tanit, but not Sopline (Tava et al. 1995).

Although much of the evidence implicates alkaloids and phenolics to be involved, little is known about the impact of volatile compounds. The impacts of on volatile compounds emitted by *F. arundinacea* and how changes in the profile of volatile compounds — specific to each germplasm — influence insect behaviour have not yet been elucidated. Therefore, the aim of the present study were to compare the profile of the volatile compounds of the Mediterranean *F. arundinacea* with their Continental counterpart

and assess the effects of endophyte *N. coenophialum* strains E^{AR542} infection on volatile compounds profile in Mediterranean and Continental germplasms. We also assessed the host selection by adults of *H. arator*, whether any alteration in the host selection is affected by volatile compounds produced by the host-specific germplasm, or affected by the *N. coenophialum* E^{AR542} infection.

Materials and Methods

Plant Samples

Seed samples of Mediterranean *F. arundinacea* germplasm either endophyte-free (ME⁻) cv. Flecha or infected with E^{AR542} (ME^{AR542}) cv. Flecha Max P[™] and seed samples of Continental *F. arundinacea* germplasm either endophyte-free (CE⁻) cv. Jesup or infected E^{AR542} (CE^{AR542}) cv. Jesup Max P[™] were obtained from Auswest Seeds (Forbes, Australia). Seed from each sample was sown in commercial-potting mix in plastic pots. The presence of E^{AR542} and its absence (E⁻) in both germplasms was confirmed using the tissue-print immunoblot technique as described in (Hahn et al. 2003). Grasses that were not of the specified endophyte status for each sample were discarded. All pots were maintained in the glass house (18–25°C; 75% RH) at Charles Sturt University (CSU, Orange, NSW) and watered uniformly until analysis.

Extraction of volatile compounds from *Festuca arundinacea* plants

Volatile compounds were extracted from 10-month old ME⁻, ME^{AR542}, CE⁻ and CE^{AR542} plants using a solid-phase microextraction (SPME) technique as previously described in (Qawasmeh et al. 2015). Briefly, an individual potted plant from each ME⁻, ME^{AR542}, CE⁻, and CE^{AR542} treatment was placed on a porous ceramic plate kept inside a standard clean laboratory desiccator connected to vacuum pump. All pots were wrapped with commercially available polyethylene sheet (Gladwrap[™]) to minimize possible contamination with compounds emanating from either the pot or the soil. Leaves of *F. arundinacea* were left exposed to the air inside the desiccator. All plant samples were incubated in the desiccator for 60 min. Volatile compounds accumulated in the headspace of the enclosed desiccator were collected by an SPME fibre (1 cm thick) coated with 75-µm-thick polydimethyl-siloxane-carboxen (Cat #: 57344-U, Supelco-Sigma-Aldrich, Sydney, Australia) mounted on SPME fibre holder (Cat #: 57330-U, Supelco-Sigma-Aldrich, Sydney, Australia). The SPME fibre was conditioned at the start of the day by inserting the needle of the holder into the injection port (250°C) of the GC–MS, exposing the fibre and allowing it to stand for one complete GC–MS run. The fibre was then retrieved into the needle of the holder. The holder was drawn out of the GC–MS port and its needle was then immediately inserted into the headspace of the desiccator through a rubber septum and the fibre was exposed to the headspace. After 60 min, the fibre was retrieved into the needle of the holder and immediately injected into the GC–MS. This time length was adequate to achieve a distinct profile of volatile compounds. Shorter time lengths gave less distinctive profile. The same fibre was used throughout the analysis.

GC–MS analysis

Volatile compounds emitted by *F. arundinacea* were determined using GC–MS (QP5050A, Shimadzu, Kyoto, Japan). Oven temperature was maintained at 40°C for 2 min and was programmed to rise to 250°C at 6°C/min rate and then to 280°C at 40°C/min rate. Temperature of the injector and the detector was maintained at 250°C and 260°C, respectively. A DB–5 ms column (30 m × 0.2 mm with 0.25 µm film thickness) was used. Helium was the carrier gas; the total-gas flow and velocity were maintained at 134.3 mL/min and 43.1 cm/sec, respectively. MS scan speed was 1000 amu/sec and the molecular masses (*M/Z*) between 70 and 700 *M/Z* were acquired. The analysis was repeated five times for each sample, each repeat was performed on a different individual grass plant from the same sample. Alkane standard solution C₈–C₂₀ (Sigma-Aldrich, Sydney, Australia) was injected in the GC-MS under the same conditions to determine the Kováts retention index (KI) for each volatile compound. KI was calculated based on the formula:

$$KI = 100n + 100 * [(LogRt_{(X)} - LogRt_{(Y)}) / (LogRt_{(Z)} - LogRt_{(Y)})]$$

where

KI = Kováts retention index; *n* = the number of carbon atoms in the smaller n-alkane;

Rt = retention time; X = unknown volatile; Y = smaller n-alkane; Z = larger n-alkane.

Collection of *Heteronychus arator*

Heteronychus arator involved in this study were collected from either the fairways of Duntryleague Golf Course, Orange [33°15'S, 149°07'E], NSW or from *Lolium perenne* paddocks in Bodalla [36°05'S, 150°03'E], NSW. The collected beetles were kept in plastic containers with slices of potato tubers and covered with soil obtained from the *L. perenne* paddocks. The container was covered with a plastic lid and stored at 20°C and RH 68%.

Four-choice bioassay

The four-choice bioassay was performed to determine the effect of sex on the host selection of *H. arator* adults. The four choices provided were ME[−], ME^{AR542}, CE[−] and CE^{AR542}. The arena for the four-choice bioassay was similar to that described in Qawasmeh et al., 2015. Briefly, a single run in the four-choice assay consisted of adult males (*n* = 8) or adult females (*n* = 8). Males and females were assays separately. Beetles were introduced to the centre of the arena individually, but at the same time, at the centre of the plastic container. The number of beetles that either 'chose' or 'not' was recorded after 20 min. Beetles remained either on the grass tillers or within 1-cm distance from the leaves were considered to have made the choice. Beetles remaining at the centre of the plastic container or those remained farther than 1 cm from grass tillers after 20 min were considered 'not choosing'. A 20-min period was determined appropriate to make these measurements by doing trial runs for 5, 10 and 15 min. The whole run was repeated 10 times involving same beetles and providing fresh grass tillers at the beginning of

each run. The location of each tillers were being randomly relocated after each run. The arena was cleaned with deionized water followed by aqueous ethanol (70%v/v) after every two runs and air-dried.

Y-tube olfactometer bioassay

The Y-tube olfactometer bioassays were performed to determine the impact of the germplasm and E^{AR542} -infection on host-selection preference of *H. arator* adults in relation to the volatiles produced. Each run in the olfactometer bioassay consisted of 8–10 beetles introduced individually, all about the same time, into the stem of the Y-tube and observed for 20 min. After 20 min the number of beetles that either 'chose' or not was recorded. Beetles moved over a 5 cm distance along one arm of the Y-tube junction were considered 'choosing'. Any beetle that neither moved towards the junction nor moved less than 5 cm distance past the junction was considered 'not choosing'. The total number of repeats for each comparison made was eight. The positions of *F. arundinacea* plants were randomly allocated on each side of the olfactometer and were swapped every second repeat to minimize potential directional bias.

Data analysis

Acquired GC–MS chromatograms were integrated using GC–MS solution software Version 1.2 (Shimadzu, Kyoto, Japan). The quantity of each volatile compound, average area under the curve (AUC), for each volatile in ME^- , ME^{AR542} , CE^- , and CE^{AR542} was determined. The percentage of each volatile compound from the total quantities of all volatile compounds within each sample was calculated. To compare the quantity of the volatile compounds between samples, their quantities in ME^{AR542} , CE^- and CE^{AR542} were expressed as percentage to those detected in ME^- .

Identification of the volatile compounds was confirmed by matching their mass spectrum with authentic standards available in the NIST mass–spectral library of GC–MS. The mass spectrum and the calculated Kováts retention index of each volatile compound detected in this study were verified with authentic standards analysed under the same chromatographic conditions described in El-Sayed (2011). In Y-tube olfactometer and four-choice bioassays, the numbers of *H. arator* 'choosing' or 'not choosing' were determined and expressed as the percentage of the total number of beetles introduced. The difference in the means was determined using χ^2 test of goodness of fit using SPSS software version 16.0.1 (IBM, Chicago, USA). Data were considered statistically significant when $P < 0.05$.

Results

Festuca arundinacea irrespective of the germplasm or endophyte status released plethora of volatile compounds. A twenty-two volatile compounds were detected in the *F. arundinacea* samples tested (Fig. 1). The identity of the volatile compounds detected in Mediterranean *F. arundinacea* plants were similar to those detected in the Continental *F. arundinacea* (volatile compounds having same retention time showed same M/Z, Fig. 1). *Neotyphodium coenophialum* E^{AR542} infection did not affect the identity of the volatile compounds in either of the germplasms.

Table 1

Relative quantities of volatile compounds from Mediterranean *Festuca arundinacea* endophyte-free (ME⁻) or infected with *Neotyphodium coenophialum* strain AR542 (ME^{AR542}) and Continental *Festuca arundinacea* endophyte-free (CE⁻) or infected with *Neotyphodium coenophialum* strain AR542 (CE^{AR542}). Parentheses indicate the quantities of the volatile compounds expressed as percentage of ME⁻ (= 100%) KI: Kováts retention index. *Rt*: retention time.

Peak No	<i>Rt</i> (min)	KI	Identification	Relative quantities			
				ME ⁻	ME ^{AR542}	CE ⁻	CE ^{AR542}
1	11.18	1024	Octanal	3.7	4.6 (117)	3.9 (100)	3.5 (90)
2	11.34	1030	2-ethylhexanol	9.9	12.3 (119)	10.9 (106)	10.3 (100)
3	13.48	1105	Nonanal	1.4	1.4 (96)	1.4 (99)	1.5 (106)
4	16.10	1203	Dodecane	7.0	7.4 (100)	7.6 (103)	6.7 (91)
5	16.19	1207	Decanal	1.3	0.7 (53)	1.3 (96)	1.4 (103)
6	16.28	1211	Pentanal	0.6	1.1 (158)	0.7 (103)	0.7 (98)
7	16.49	1220	(<i>Z</i>)-2-hexen-1-ol	26.7	27.8 (99)	28.6 (102)	25.3 (90)
8	16.79	1232	Butyl hexanoate	6.9	7.2 (100)	7.3 (102)	6.8 (95)
9	18.22	1288	1,6-octadien-1-ol	0.7	0.6 (88)	1.0 (144)	0.8 (109)
10	18.65	1304	Hexanal	0.5	0.8 (169)	0.6 (114)	0.7 (134)
11	18.73	1307	Undecanal	0.4	0.5 (118)	0.4 (92)	0.5 (109)
12	20.12	1361	Hexanol	1.0	1.1 (108)	1.2 (119)	0.9 (95)
13	20.92	1390	Undecane	0.5	0.5 (92)	0.6 (100)	0.8 (136)
14	21.25	1402	Tetradecane	6.0	5.9 (93)	6.7 (105)	5.1 (80)
15	22.25	1454	1-octen-3-ol	8.3	13.2 (150)	10.3 (117)	16.8 (193)
16	22.37	1460	(<i>Z</i>)-2-octen-1-ol	0.7	1.0 (149)	0.7 (107)	1.1 (157)
17	22.59	1471	Clovene	0.6	0.8 (114)	0.7 (98)	1.0 (156)
18	23.10	1496	Decane	0.6	0.6 (93)	0.6 (100)	0.6(95)
19	23.25	1503	Butylated hydroxytoluene	18.6	7.8 (40)	8.2 (42)	11.7 (60)
20	23.92	1536	Benzylhexanoate	1.3	1.3 (96)	1.4 (105)	1.1 (81)
21	25.07	1589	Ethyl-3-hydroxypentanoate	2.8	2.9 (98)	5.4 (184)	2.7 (92)

Peak No	Rt (min)	KI	Identification	Relative quantities			
				ME ⁻	ME ^{AR542}	CE ⁻	CE ^{AR542}
22	25.32	1601	Hexylhexanoate	0.7	0.4 (61)	0.5 (71)	0.5 (78)

The relative quantity of each volatile compound varied in each plant sample whether being a ME⁻, CE⁻, ME^{AR542} or CE^{AR542}. Octanal, 2-ethylhexanol, dodecane, (Z)-2-hexen-1-ol, butyl hexanoate, (Z)-3-hexen-1-ol, 1-octen-3-ol, and butylated hydroxytoluene were the major compounds detected in the tested *F. arundinacea* samples. These compounds constituted almost 80% of the total volatile compounds detected (Table 1).

The effect of the germplasm on the quantity of volatile compounds was examined by comparing the quantity of volatile compounds detected in ME⁻ with those detected in CE⁻. Compared with ME⁻, 1,6-octadien-1-ol, hexanal, hexanol, 1-octen-3-ol and ethyl-3-hydroxypentanoate were greater in CE⁻ by 44, 14, 19, 17 and 84%, respectively. However, butylated hydroxytoluene and hexylhexanoate were lesser in CE⁻ by 60 and 39%, respectively. The remaining volatile compounds detected in CE⁻ were either equal or varied by < 10% to the corresponding compounds detected in ME⁻.

Impact of the infection by *N. coenophialum* strain AR542 on the quantity of volatile compounds was examined in the Mediterranean and Continental germplasms. In the Mediterranean germplasm (the ME^{AR542}), octanal, 2-ethylhexanol, pentanal, hexanal, undecanal, 1-octen-3-ol, (Z)-2-octen-1-ol and clovene were greater by 17, 19, 58, 69, 18, 50, 49, and 14%, respectively, compared with ME⁻. In contrast, decanal, 1,6-octadien-1-ol, butylated hydroxytoluene and hexylhexanoate detected in ME^{AR542} were less by 47, 12, 60 and 39%, respectively than those detected in ME⁻ (Table 1). Other volatile compounds detected in ME^{AR542} were either equal or varied by < 10% to the corresponding compounds detected in ME⁻. In the Continental germplasm, hexanal, undecanal, undecane, 1-octen-3-ol, (Z)-2-octen-1-ol, clovene and butylated hydroxytoluene detected in CE^{AR542} were higher by 17, 19, 36, 65, 48, 59, and 43%, respectively than CE⁻. In contrast, dodecane, (Z)-2-hexen-1-ol, 1,6-octadien-1-ol, hexanol, tetradecane, benzylhexanoate and ethyl-3-hydroxypentanoate detected in CE^{AR542} were lower by 11, 11, 24, 20, 23, 23 and 50%, respectively than those detected in CE⁻. Other volatile compounds in CE^{AR542} varied by ≤ 10% compared with their corresponding compounds detected in CE⁻.

The effect of sex of *H. arator* adults on host selection was determined using the four-choice bioassay. The percentage of the numbers of *H. arator* adult males choosing one of the choices ME⁻, ME^{AR542}, CE⁻ or CE^{AR542} was not significantly different to the number of the adult females choosing any of the respective choices (Table 2).

Table 2

Host selection of adult males and females of *Heteronychus arator* to Mediterranean *Festuca arundinacea* either endophyte-free (ME⁻) or infected with *Neotyphodium coenophialum* strains AR542 (ME⁺) and Continental *Festuca arundinacea* either endophyte-free (CE⁻) or infected with *Neotyphodium coenophialum* strains AR542 (CE⁺) assessed by four-choice assay. * Average percentage of beetle numbers expressed as Mean $\pm$ SEM.

<i>Festuca arundinacea</i>	ME ⁻	ME ^{AR542}	CE ⁻	CE ^{AR542}
Males	18.6 $\pm$ 3.9*	12.9 $\pm$ 5.2	34.3 $\pm$ 5.1	24.3 $\pm$ 4.5
Females	21.4 $\pm$ 5.1	10.0 $\pm$ 5.0	34.3 $\pm$ 6.4	17.1 $\pm$ 6.7

The impact of the germplasm on the host selection of *H. arator* adults was determined using two-choice assay olfactometer. The percentage of *H. arator* adults choosing ME⁻ (60%) was significantly greater ($\chi^2 = 10$, *d.f.* = 1, *P* < 0.002) than those choosing CE⁻ (30%) (Fig. 2A). When comparing ME^{AR542} with CE^{AR542}, the percentage of the numbers of *H. arator* adults choosing ME^{AR542} (10%) was significantly lesser ($\chi^2 = 26.67$, *d.f.* = 1, *P* < 0.05) than adults choosing CE^{AR542} (50%).

The impact of *N. coenophialum* E^{AR542} infection on the host selection of *H. arator* adults was determined also using two-choice assay. The percentage of *H. arator* adults choosing ME^{AR542} (31%) was not significantly different ($\chi^2 = 3.66$, *d.f.* = 1, *P* < 0.056) compared with adults choosing ME⁻ (48%) (Fig. 2B). The percentage of *H. arator* adults choosing CE^{AR542} (51%) was not significantly different ($\chi^2 = 1.33$, *d.f.* = 1, *P* < 0.249) compared with adults choosing CE⁻ (40%).

Discussion

Endophyte infection is widely accepted to be a natural and cost-effective method to enhance persistence and productivity of pasture grasses, by increasing their resistance to herbivorous insects (Kuldau and Bacon 2008; Malinowski and Belesky 2006). Many studies have considered the alkaloids produced during the endophyte–grass association as the primary factors mediating this resistance (Ball et al. 2006; Bush et al. 1997; Hunt and Newman 2005; Popay and Jensen 2005). The impact of fungal endophyte infection on the volatile compounds is not yet fully explained, although these compounds play a central role in modulating grass–insect relationships either directly by deterring herbivorous insects or indirectly by attracting their natural enemies (Degenhardt 2009; Qawasmeh et al. 2015; Ton et al. 2007). In this study, the impact of *N. coenophialum* E^{AR542} on the quantity and identity of the volatile compounds produced by the Mediterranean and Continental germplasms *F. arundinacea* were examined.

In this study, E^{AR542} infection influenced the relative quantities of the volatile compounds emitted from either Mediterranean or Continental *F. arundinacea*. For example, in the Mediterranean germplasm, this influence was characterised by either an increase or a decrease in the relative quantities of different volatile compounds. Quantities of 2-ethylhexanol and 1-octen-3-ol in ME⁻ were 9.9 and 8.3%,

respectively. Their quantities in ME^{AR542} were increased by 12.3 and 13.2%, respectively. In contrast, the relative quantities of decanal and butylated hydroxytoluene in ME⁻ were 1.3 and 18.6%, respectively. Their relative quantities in ME^{AR542} were reduced by 0.7 and 7.8%, respectively (Table 1). These findings are consistent with those of Yue et al. (2001), who reported that E^{WT} infection variably altered the relative quantities of the volatile compounds, e.g., heptanal, nonanal, decanal, 2-octanone, (*Z*)-3-hexen-1-ol acetate produced by *F. arundinacea* (clone FD4N) plants.

Tomato plants produce wide range of volatile compounds, mainly β -thujene, α -phellandrene, which constitute 85% of the total volatiles produced (Jallow et al. 2008). Their quantities and the other volatiles present in tomato are significantly reduced by *Acremonium strictum* E^{WT} infection. The only exceptions were β -caryophyllene, representing < 5% of the total volatiles produced, which is significantly increased by *Acremonium strictum* E^{WT} infection and α -terpinene, representing < 1% of the total volatiles produced, which levels has not significantly affected (Jallow et al. 2008). The reduction in the quantity of the major volatile compounds in tomato due to *Acremonium strictum* E^{WT} infection has been associated with higher numbers of *Helicoverpa armigera* attracted to the plant (Jallow et al. 2008). The alteration in the profile of volatile compounds observed in *F. arundinacea* E^{WT} has been suggested to have little impact on influencing grass–insect relationships (Yue et al. 2001). This suggestion has been based on the observation that the levels of the volatile compounds (*E*)- β -ocimene and (*Z*)- β -ocimene/(*E*)- α -ocimene in *F. arundinacea* E^{WT} induced after jasmonic acid treatment were similar to E⁻. The findings reported in this study that *H. arator* adults, in the Y-tube olfactometer bioassay, did not prefer ME⁻ over ME^{AR542} and also did not prefer CE⁻ over CE^{AR542} as hosts confirm Yue et al. (2001) findings. However, variation in the quantities of the volatile compounds due to E^{AR542} infection reported in this study and in Yue et al. (2001) is likely to be a part of the mutualism established between *F. arundinacea* and *N. coenophialum*, where the fungi alters the profiles of volatile compounds and the production of some secondary compounds to achieve optimal metabolic changes as suggested by Clay & Schardl (2002) and Schulz et al. (1999).

In the Y-tube olfactometer bioassay, in which host selection by adults of *H. arator* was mediated only by olfactory cues directed by the volatiles, there was no difference in host selection (ME⁻ vs ME^{AR542}) of *H. arator* adults. However, in the four-choice assay, where mechanoreception and chemoreception cues were involved, ME⁻ was significantly preferred as a host by the adults of *H. arator*, irrespective of their sex and similar findings were observed for the continental germplasm (Figs. 2 and 3). The discrepancy between the host selection of *H. arator* adults recorded using olfactometer and those recorded using four-choice assay indicates *H. arator* adults do not discriminate between the hosts solely based on the volatiles emitted. Instead, visual and leaf-chemical cues are also involved by *H. arator* to select the appropriate host. Given that several Coleoptera species are capable of selecting their host via visual cues (Butkewich and Prokopy 1997; Cavaletto et al. 2020; Lyu et al. 2018), it is reasonable to argue that *H. arator* selected their host in the four–choice assay based on the visual and leaf-chemical cues, although volatile cues may have played a role.

Several morphological and physiological differences between Mediterranean and Continental germplasms have been recognized (Assuero et al. 2002; Hill et al. 1985). For example; the Mediterranean cultivar (Maris Kasba) exhibits lower shoots and dead tissues dry weight but higher tiller number and root–shoot ratio compared with the Continental cultivars (Grasslands Advance, El Palenque) as a mechanism of adaptation to water deficit stress (Assuero et al. 2002). In addition, Hill et al. (1985) reported slower growth rates in the Mediterranean germplasm compared with the Continental germplasm when both are subject to an identical growth conditions. In this study, we provided additional evidence that the Mediterranean germplasm is different to the Continental germplasm in relation to the quantities of volatile compounds produced. Volatile compounds quantities in Mediterranean (ME[−]) germplasm were not identical to those detected in Continental (CE[−]) germplasm (Table 1). These quantities were affected by E^{AR542} infection but the effects were different in Mediterranean compared with Continental. Whether the change in volatile compounds quantities imposes any agricultural or ecological impacts require further investigation.

Declarations

Author contributions

AQ conducted all the reported experimental work and wrote the manuscript and WW and RA improved the manuscript.

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Figures

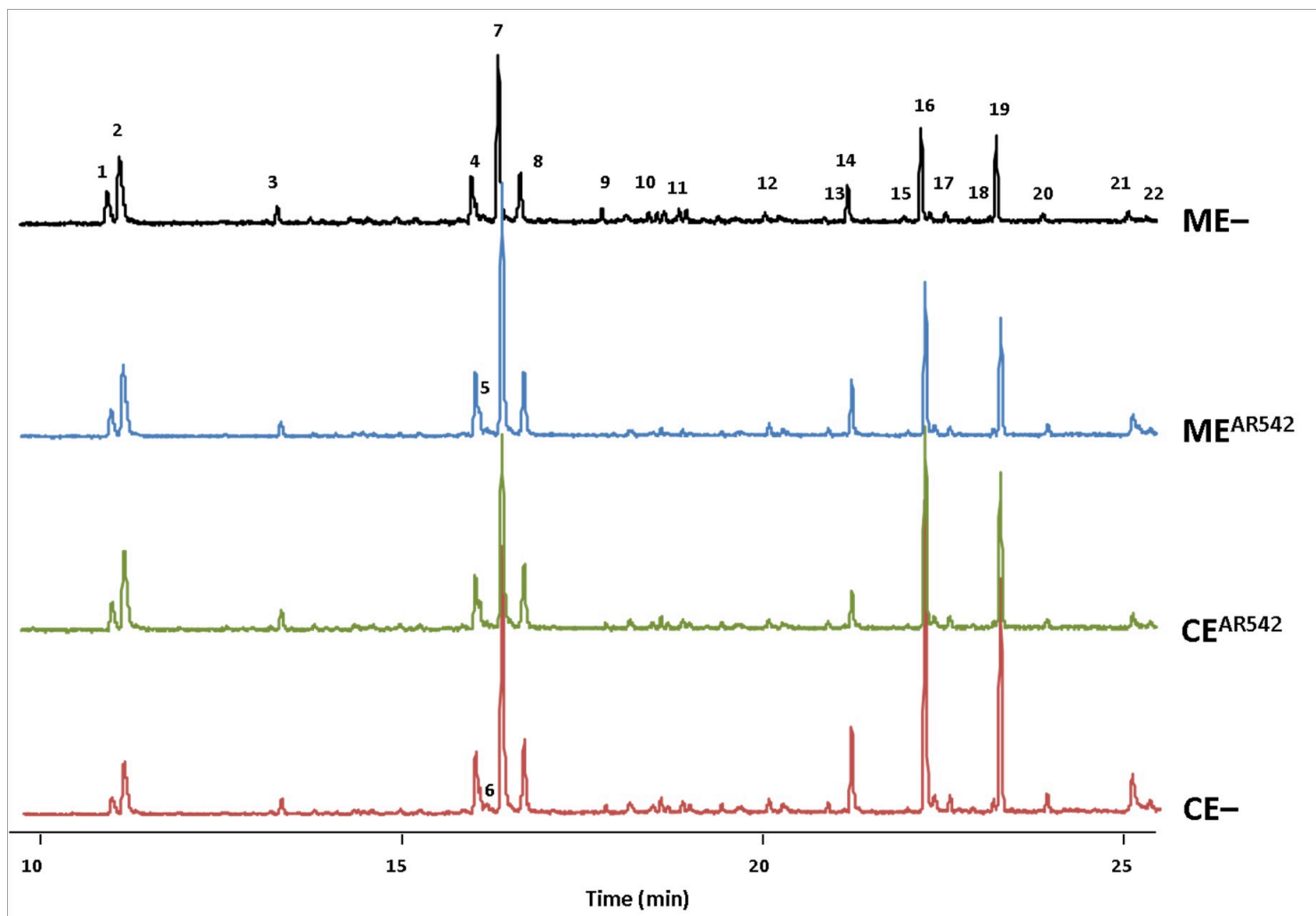


Figure 1

Representative total ion chromatograms showing the volatile compounds extracted from Mediterranean *Festuca arundinacea* either endophyte-free (ME⁻) or infected with *N. coenophialum* strains AR542 (ME^{AR542}) and Continental *Festuca arundinacea* either endophyte-free (CE⁻) or infected with *N. coenophialum* strains AR542 (CE^{AR542}). Each numbered peak represents a volatile oil listed in Table 1.

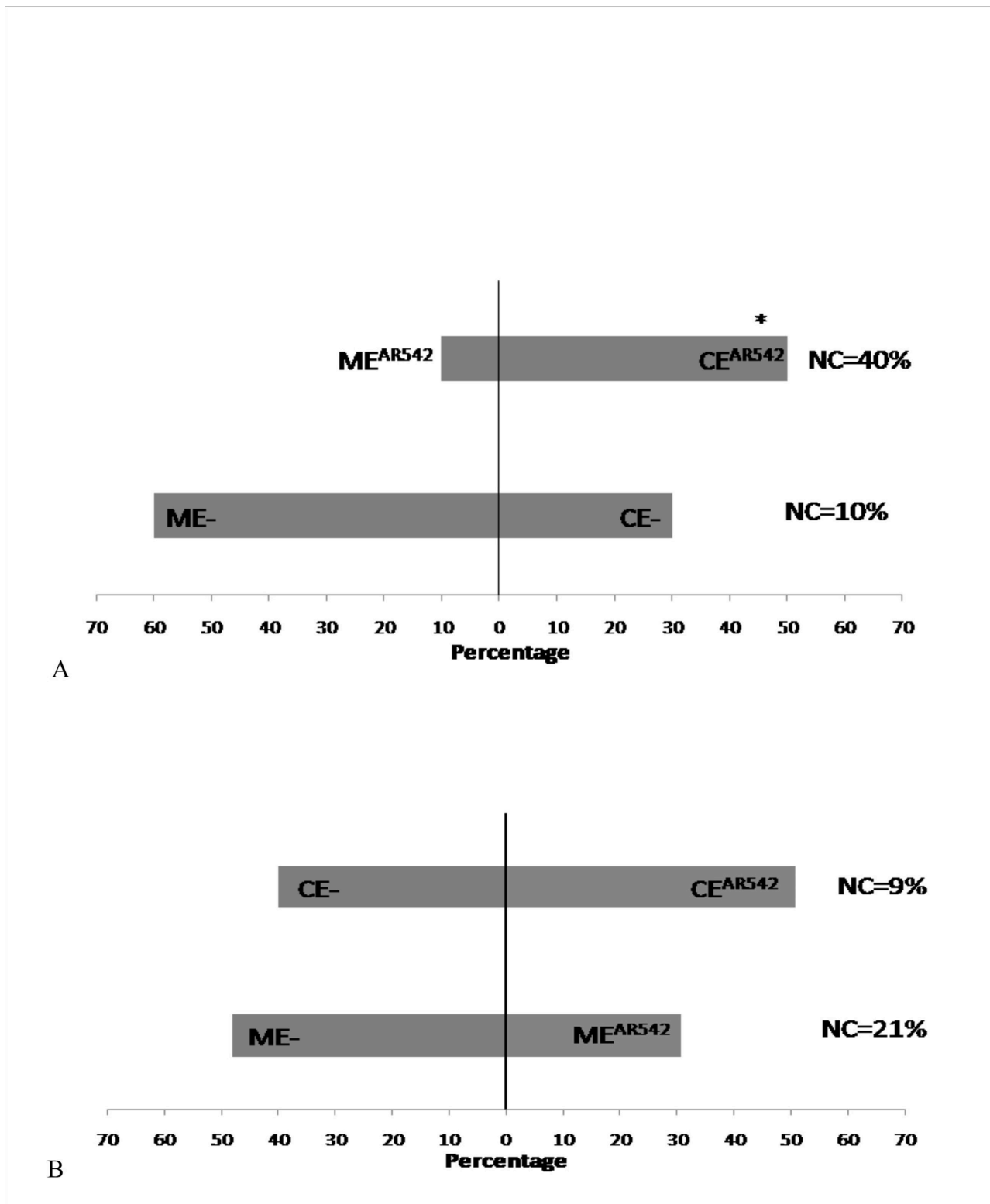


Figure 2

Effect of the germplasm (A) and *Neotyphodium coenophialum* strain AR542 (B) on the host-selection of *H. arator* adults assessed using a Y-tube olfactometer ME⁻: Mediterranean *Festuca arundinacea* endophyte-free, ME^{AR542}: Mediterranean *Festuca arundinacea* infected with *N. coenophialum* strains AR542, CE⁻: Continental *Festuca arundinacea* endophyte-free, CE^{AR542}: Continental *Festuca*

arundinacea infected with *N. coenophialum* strains AR542. * Data were considered statistically significant if $P < 0.05$ using χ^2 test goodness of fit. NC = % of beetles that did not make a choice.