Soil Microorganisms Alleviate the Allelochemical Effects of a Thyme Monoterpene on the Performance of an Associated Grass Species

Bodil K. Ehlers*
Institute of Biology, University of Southern Denmark, Odense, Denmark

Abstract

Background: Plant allelochemicals released into the soil can significantly impact the performance of associated plant species thereby affecting their competitive ability. Soil microbes can potentially affect the interaction between plant and plant chemicals by degrading the allelochemicals. However, most often plant-plant chemical interactions are studied using filter paper bioassays examining the pair-wise interaction between a plant and a plant chemical, not taking into account the potential role of soil microorganisms.

Methodology/Principal findings: To explore if the allelopathic effects on a grass by the common thyme monoterpene “carvacrol” are affected by soil microorganisms. Seedlings of the grass Agrostis capillaris originating from 3 different thyme sites were raised in the greenhouse. Seedlings were grown under four different soil treatments in a 2*2 fully factorial experiment. The monoterpene carvacrol was either added to standard greenhouse soil or left out, and soil was either sterilized (no soil microorganisms) or not (soil microorganisms present in soil). The presence of carvacrol in the soil strongly increased mortality of Agrostis plants, and this increase was highest on sterile soil. Plant biomass was reduced on soil amended with carvacrol, but only when the soil was also sterilized. Plants originating from sites where thyme produces essential oils containing mostly carvacrol had higher survival on soil treated with that monoterpene than plants originating from a site where thyme produced different types of terpenes, suggesting an adaptive response to the locally occurring terpene.

Conclusions/Significance: The study shows that presence of soil microorganisms can alleviate the negative effect of a common thyme monoterpene on the performance of an associated plant species, emphasizing the role of soil microbes in modulating plant-plant chemical interactions.

Introduction

Allelochemicals released into the soil by plants can affect the performance of interacting plant species [1]. Allelochemicals can act as selective agents driving adaptation in associated plants species to cope with the compounds released by their “chemical neighbor” [2,3,4]. In this context, the soil microbial community can potentially affect the interaction between plants and plant chemicals as soil microorganisms may degrade allelochemicals after entering the soil [5]. Most studies examining the effect of plant allelochemicals use bioassays where the effect of a chemical on seed germination and growth is tested in isolated petri dishes. By doing so these studies do not examine the effects of soil microorganisms [5].

Terpenes are the most common group of plant secondary compounds and are produced by a vast amount of plants – both herbs and trees [6,7]. These compounds can have both inhibitory and stimulating effect on a number of associated organisms including plants, herbivores and microorganisms [7].

Many aromatic plants of the family Lamiaceae produce monoterpenes as a main constituent of their essential oils. These monoterpenes are known for their antimicrobial activity [8,9,10] mainly through their growth inhibitor effect on bacteria and fungi. However, it has been shown that some bacteria can decompose terpenes and use some of them as a carbon source [8,9,10]. One genus well known for its production of monoterpenes is Thymus. In some thyme species distinct chemotypes can be identified, where an individual plant often produces a single specific dominant monoterpene making up 60-80% of the essential oils that confer thyme plants their characteristic smell [11]. Thyme monoterpenes enter the soil via leaf leachates, and are known to affect the performance of associated plants [12,13]. In general, the monoterpenes reduce seed germination and plant growth, but it has been shown that associated plants can adapt to their specific local thyme monoterpene [3,14]. Studies on the impact of thyme monoterpenes on plant growth have been performed using non-sterile soil, either standard greenhouse soil where monoterpenes were manually added, or soil collected from natural sites. The
documented effect of the thyme monoterpenes on plant performance therefore included the interaction with the soil microbial community.

The main purpose of this study was to examine if the effect of a single common thyme monoterpane - carvacrol - on the performance of an associated grass, was affected by the presence of soil microorganisms. In Denmark, the grass Agrostis capillaris is commonly found in dry grasslands where it co-occurs with T. pulegoides. Thymus pulegoides produces the monoterpane “carvacrol” as main constituent of its essential oil, and this monoterpane can make up between 50–80% of the total constituent of the oil [15]. Agrostis capillaris is also found co-occurring with another thyme species, T. serpyllum. The essential oil of T. serpyllum is not as pure as in e.g. T. pulegoides, and usually consists of a mix of 2-3 different terpenes where carvacrol is not a dominant component ([11], Keefoverring, Grøndahl & Ehlers unpubl. data).

Materials and Methods

Experimental design

Plants originating from different maternal seed families of Agrostis capillaris were grown in individuals pots in a combination of two different types of soil treatments: Soil that was either treated with the thyme monoterpane “carvacrol” or not, and on soil that was either sterilized or not. Plants were grown in a fully factorial design in the greenhouse.

Plant material

Agrostis seeds were collected in August 2008 from different maternal plants growing at three natural sites where they co-occurred with thyme plants, (often growing in the middle of thyme tufts). At two sites (hereafter named TP1 and TP2). Agrostis grows together with Thymus pulegoides producing the monoterpane carvacrol as the main constituent of its oil [15]. At the third site (TS1), Agrostis grows with T. serpyllum, which produces oil that is a mix of different terpenes, dominated by sesquiterpens and β-caryophyllene (Keefoverring, Grøndahl & Ehlers unpubl. data). Collected seeds were stored in paper bags in a dry room until their use the following spring.

Using maternal seed families rather than bulk samples of seeds allow testing for differences in performance among seed families within populations. Albeit confounded with maternal effects [16], detection of significant differences among families suggests presence of genetic variation for performance.

Soil preparation

Standard greenhouse soil (Pindstrup no 1) was steam sterilized and kept in sealed plastic bags until use. A standard fertilizer mix (N-P-K) was subsequently added to both sterilized and non-sterilized soil, corresponding to 0.078 g N, 0.039 g P, and 0.13 g K per litre soil.

Soil containing the thyme monoterpane carvacrol was prepared by adding 40 μl of liquid pure carvacrol (Sigma Aldrich)/100 g soil (dry weight). This concentration of carvacrol (approximately 400 μg g⁻¹ soil) is within the high range of monoterpane concentrations that may be found under natural field conditions [14,17]. The monoterpane was added to the soil in the following way: Liquid carvacrol was mixed in petri dishes with filter paper (Filtrak paper sheet, 17.95 g m⁻²) cut in pieces of approximately 1 cm² and sealed with plastic film for 24 h after which all liquid had soaked into the filter papers. Filter paper was then mixed thoroughly into soil using one single container of soil per treatment. Soil with filter paper was sealed with plastic and left for another 24 h to homogenize the concentration of carvacrol before adding soil to individual pots.

Germination and transplantation

Agrostis seeds were sown in germination trays containing standard greenhouse soil (Pindstrup no 1). Two weeks after germination, seedlings were transplanted to individual pots (10 cm in diameter). Four seedlings from each maternal family were transplanted to each of the four combinations of soil treatment (soil with and without monoterpane on sterile and non-sterile soil), - each plant grown alone in individual pots, yielding 16 individual plants per maternal family. Care was taken to choose seedlings of similar size in all treatment combinations.

There was variation in the number of maternal seed families from which 16 equal sized seedlings could be obtained. The number of maternal families from each population was 18 (sites TP1 and TP2) and 10 (site TS1) yielding a total of 736 seedlings in individual pots (184 for each of the four soil treatment combinations).

Plants were grown in an unheated glasshouse without addition of plant growth light, so the light range followed the natural photoperiod of the growing season from spring to late summer.

Plants were randomized twice a week to avoid any position effects, and all surviving adult plants were harvested three months after transplant at the end of August 2009. Due to damage of roots when removing soil, root biomass was not included in the analysis.

Dry weight of aboveground biomass of each plant was assessed using a milligram precision balance.

Data analysis

Survival of plants was analyzed using a logistic regression to test for the effects of monoterpane, soil microorganisms, populations, maternal family (nested within populations), and their interactions. The analysis was performed assuming a binomial distribution of survival data as implemented in the glm function of the statistical software R (version 2.10.1, R Development Core Team, 2008). Tests of hypothesis regarding the effects of soil treatments, population and their interaction were performed using likelihood ratio tests (LRTs) between nested models and assuming that LRTs statistics where Chi-square distributed.

ANOVA was used to examine for the effect of monoterpane, soil microorganisms, population, maternal family (nested within population), and their interactions on the biomass of plants. The analysis of variance was performed using the software package JMP version 8.0 [18]. This package uses the REML method for variance component and parameter estimation. This method handles naturally unbalanced design and does not rely on approximation for F-test.

Results

Survival

Seedling mortality occurred within the first three weeks after transplantation.

Presence of the thyme monoterpane in the soil greatly reduced the survival of Agrostis plants and this effect was reinforced in the absence of soil microorganisms (Fig. 1, Table 1 - interaction between terpene and soil microorganisms). Survival ranged from over 90% in soil without terpene (both sterile and non-sterile soil) to between 20–40% on non-sterile soil treated with monoterpane to as low as 3–8% on sterile soil treated with monoterpane (Fig. 1).

A significant population effect showed that survival differed among plants originating from different sites. Agrostis plants from the T. serpyllum site had generally a lower survival rate compared to plants from either of the two T. pulegoides sites, but this difference
was only present on soil treated with the \textit{T. pulegioides} monoterpene (Fig. 1, compare black triangles with grey symbols on T-soil vs No T soil).

The analysis also revealed a significant variation among maternal seed families (nested within populations) for survival rates. The mortality on sterile soil amended with monoterpene was so high that very little power was left to test if variation among maternal families in survival also varied with presence/absence of soil microbes.

Biomass

The ANOVA revealed a significant effect of both terpene, soil microorganisms and their interaction on the biomass of \textit{Agrostis}.

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|l|}
\hline
Source & df & Sum of Squares & F \\
\hline
Monoterpene & 1 & 14.97 & 14.78*** \\
Soil & 1 & 9.60 & 9.49** \\
Population & 2 & 0.68 & 0.34 \\
Family (Population) & 43 & 100.06 & 2.30*** \\
Monoterpene*Soil & 1 & 4.55 & 4.49* \\
Monoterpene*Population & 2 & 0.58 & 0.29 \\
Soil*Pop & 2 & 0.13 & 0.06 \\
Monoterpene*Soil*Population & 2 & 0.46 & 0.23 \\
Residual & 373 & 377.6 & \\
\hline
\end{tabular}
\caption{Summary of ANOVA on plant biomass.}
\end{table}

Table 2. Summary of ANOVA on plant biomass.

The addition of monoterpene to the soil reduced biomass of plants but only on sterile soil (Table 2; Fig. 2). The effect of maternal family indicates a variation in biomass among the different seed families within populations. As for survival, the high mortality of plants on sterile soil containing monoterpene excluded the possibility to test if differences in biomass among maternal families varied among sterile and non-sterile soil.

Discussion

The main finding of this study is that the soil microorganisms present in the soil can significantly affect the outcome of the plant-plant chemical interaction between a grass and the monoterpene produced by its thyme neighbor plant. Presence of the \textit{T. pulegioides} monoterpene carvacrol in the soil strongly reduced survival of \textit{Agrostis} plants, and mortality was highest when soil microorganisms were not present. In fact, only between 3% (site TS) and 8% (site TP2) of the plants survived the monoterpene treatment when the soil was sterile, compared to survival rates ranging between 20 and 40% when the soil was not sterilized. Survival was uniformly high (>90%) on sterile and non-sterile soil without monoterpene (Fig. 1).

Presence of carvacrol in the soil also reduced biomass of plants, but only when plants grew on sterile soil. There was no difference in biomass of plants growing on soil with and without addition of monoterpene when the soil was not sterilized (Fig. 2). Taken together, these results suggest that the effect of monoterpene on plant survival and biomass is mediated by soil microorganisms that can either enhance or suppress the toxic effect of the monoterpene.
References

1. Inderjit (1996) Plant phenolics in allelopathy. The Botanical Review 62: 186–202.

2. Callaway RM, Aschehoug ET (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. Science 290: 521–523.

3. Ehlers BK, Thompson D (2004) Do co-occurring plant species adapt to one another? The response of Bromus erectus to the presence of different Thymus vulgaris chemotypes. Oecologia 141: 511–518.

4. Vivanco JM, Bais HP, Sturmitz FR, Theilen GC, Callaway RM (2004) Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion. Ecology Letters 7: 285–292.

5. Kaur H, Kaur R, Kaur S, Baldwin IT, Inderjit (2009) Taking Ecological Function Seriously: Soil Microbial Communities Can Obviate Allelopathic Effects of Released Metabolites. PLoS ONE 4: e7100.

6. Gershenzon J, Dudareva N (2007) The function of terpene natural products in the natural world. Nat Chem Biol 3: 408–414.

7. Langenheim JH (1994) Higher plant terpenoids: A phytocentric overview of their ecological roles. J Chem Ecol 20: 1223–1229.

8. Kalemba D, Kunicka A (2003) Antibacterial and Antifungal Properties of Essential Oils. Current Medicinal Chemistry 17.

9. Vokou D, Chalkos D, Karamanlidou G, Yiangou M (2002) Activation of Soil Respiration and Shift of the Microbial Population Balance in Soil as a Response to Lavandula stoechas Essential Oil. Journal of chemical ecology 28: 735–768.

10. Vokou D, Margaris NS (1981) Decomposition of terpenes by soil microorganisms. Pedobiologia 31.

11. Stahl-Biskup E (2002) Essential oil chemistry of the genus Thymus – a global view. In: Stahl-Biskup E, Saez F, eds. Thyme: The genus Thymus London: Francis & Taylor. pp 75–124.

12. Tarayre M, Thompson JD, Escarré J, Linhart YB (1995) Introduction in the inhibitory effects of Thymus vulgaris (Labiatae) monoterpenes on seed germination. Oecologia 101: 110–118.
13. Vokou D, Dourdi P, Blionis GJ, Halley JM (2003) Effects of Monoterpenoids, Acting Alone or in Pairs, on Seed Germination and Subsequent Seedling Growth. Journal of chemical ecology 29: 2281–2301.

14. Grøndahl E, Ehlers BK (2008) Local adaptation to biotic effects: Reciprocal transplants of species associated with aromatic Thymus pulegioides and T. seryllum. J Ecol 96: 981–992.

15. Grøndahl E, Keefover-Ring K, Ehlers BK (2008) New 4-thuyanol chemotype detected in large thyme (Thymus Pulegioides L.) growing wild in Denmark. J Ess Oil Res 20: 45–47.

16. Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. SunderlandMass.: Sinauer. xvi, 980 p.

17. White CS (1991) The role of monoterpenes in soil nitrogen cycling processes in ponderosa pine. Biogeochem 12: 43–68.

18. SAS Institute I (2008) JMP Users Guide. Cary, NC: SAS Institute Inc.

19. Jensen C, Ehlers B (2010) Genetic variation for sensitivity to a thyme monoterpane in associated plant species. Oecologia 162: 1017–1025.

20. Wilt FM, Miller GC, Everett RL, Hackett M (1993) Monoterpenic concentrations in fresh, senescent, and decaying foliage of singleleaf pinyon (Pinus monophylla Torr. & Frem.: Pinaceae) from the western Great Basin. Journal of Chemical Ecology 19: 185–194.

21. Pavolainen L, Käunonen V, Smolander A (1998) Inhibition of nitrification in forest soil by monoterpenes. Plant Soil 205: 147–154.

22. Bever JD (2007) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. The New phytologist 157: 465–473.

23. Van der Putten WH, Vet LEM, Harvey JA, Wockers FL (2001) Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. Trends in ecology & evolution (Personal edition) 16: 547–554.

24. Callaway RM, Thelen GC, Rodriguez A, Holben WE (2004) Soil biota and exotic plant invasion. Nature 427: 731–733.

25. Inderjit (2003) Soil microorganisms: An important determinant of allelopathic activity. In: Lambers H, Colmer T, eds. Root Physiology: from Gene to Function: Springer Netherlands. pp 227–236.

26. Inderjit, Pollock JL, Callaway RM, Holben W (2008) Phytotoxic Effects of (6)-Catechin In vitro, in Soil, and in the Field. PLoS ONE 3: e2536.

27. Perry L, Thelen G, Ridenour W, Callaway R, Paschke M, et al. (2007) Concentrations of the Allelochemical (±)-Catechin In Geranium maculatum; Soils. Journal of chemical ecology 33: 2337–2344.