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Induced defenses change the chemical composition of pine seedlings and influence meal properties of the pine weevil *Hylobius abietis*

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ABSTRACT

The defense of conifers against phytophagous insects relies to a large extent on induced chemical defenses. However, it is not clear how induced changes in chemical composition influence the meal properties of phytophagous insects (and thus damage rates). The defense can be induced experimentally with methyl jasmonate (MeJA), which is a substance that is produced naturally when a plant is attacked. Here we used MeJA to investigate how the volatile contents of Scots pine (*Pinus sylvestris* L.) tissues influence the meal properties of the pine weevil (*Hylobius abietis* (L.)). Phloem and needles (both weevil target tissues) from MeJA-treated and control seedlings were extracted by *n*-hexane and analyzed by two-dimensional gas chromatography-mass spectrometry (2D GC-MS). The feeding of pine weevils on MeJA-treated and control seedlings were video-recorded to determine meal properties. Multivariate statistical analyses showed that phloem and needle contents of MeJA-treated seedlings had different volatile compositions compared to control seedlings. Levels of the pine weevil attractant (+)-α-pinene were particularly high in phloem of control seedlings with feeding damage. The antifeedant substance 2-phenylethanol occurred at higher levels in the phloem of MeJA-treated than in control seedlings. Accordingly, pine weevils fed slower and had shorter meals on MeJA-seedlings. The chemical compositions of phloem and needle tissues were clearly different in control seedlings but not in the MeJA-treated seedlings. Consequently, meal durations of mixed meals, i.e. both needles and phloem, were longer than phloem meals on control seedlings, while meal durations on MeJA seedlings did not differ between these meal contents. The meal duration influences the risk of girdling and plant death. Thus our results suggest a mechanism by which MeJA treatment may protect conifer seedlings against pine weevils.

1. Introduction

The pine weevil (*Hylobius abietis* (L.)) is a severe pest of conifer seedlings in Europe during the first years after plantation (Långström and Day, 2004; Orlander and Nilsson, 1999). The damage is caused by the adult weevils' feeding on the stem bark of the seedlings, but they also feed on needles (Fedderwitz et al., 2014).

Numerous studies have shown that the chemical defenses of conifers influence the behavior of the phytophagous insects associated with them (Erbilgin et al., 2006; Heijari et al., 2008; Miller et al., 2005). In conifer bark the main chemical defenses against insect herbivores are terpenes and phenolics (Franceschi et al., 2005). The volatile monoterpenes constitute almost half of the oleoresin produced by conifers (Lewinsohn et al., 1991), and in addition there are minor amounts of the semi-volatile sesquiterpenes. Volatile terpenes may strongly affect pine weevil behavior (Björklund et al., 2005). The volatile contents of tissues of Scots pine (*Pinus sylvestris* L.) is usually dominated by two monoterpenes (Sjödin et al., 2000): α-pinene, a pine weevil attractant (Nordlander, 1990); and (+)-3-carene, a pine weevil deterrent (Lundborg et al., *submitted*).

The induced defense is under natural conditions expressed only when the plant perceives cues indicating the presence of potential attackers whereas constitutive defenses are permanently present

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(Karban and Baldwin, 1997; Stamp, 2003). However, the induced defenses can be triggered experimentally through exogenous application of chemical elicitors such as methyl jasmonate (MeJA) (Fäldt et al., 2003; Huber et al., 2005; Martin et al., 2002; Zulak et al., 2009). Exogenous application of MeJA and insect feeding provoke quite similar chemical responses in plants (Franceschi et al., 2005; Rohwer and Erwin, 2008). Treatment of conifer seedlings with MeJA can reduce feeding by the pine weevil (Heijari et al., 2005), and recently it was shown that MeJA application at the nursery stage can provide protection to seedlings over two seasons in the field (Zas et al., 2014). Thus, MeJA treatment has emerged as an attractive alternative to protect conifer seedlings against pine weevils.

Most phytophagous insects, including the pine weevil (Fedderwitz et al., 2014, 2015, 2016), feed in bouts or clusters of ingestion divided by intervals during which no feeding occurs, so called discrete meals (Simpson, 1995). Meals may be described in terms of their size (i.e. amount of tissue consumed) and duration, and these measures can then also be used to calculate the average feeding rate (Simpson, 1995). Changes that influence these measures can provide robust quantitative indications of insects' responses to variations in food quality (Colasurdo et al., 2007; Daoust et al., 2010; Despland et al., 2011; Wright et al., 2003).

Here we attempt to determine which induced changes in the chemical composition of Scots pine seedlings that cause the previously shown reduction in pine weevil feeding damage. The feeding behavior was analyzed at a temporal resolution of meals to be able to determine how the changes in chemical composition influence the mechanisms regulating feeding. We performed a sequence of experiments (Fig. 1) to determine the effects of MeJA treatment, and pine weevil feeding, on the seedlings' chemical contents in tissues (phloem and needles), and emissions from entire seedlings, as well as the effects of the treatments on the feeding behavior of the pine weevil.

2. Results

2.1. Effects of MeJA treatment and weevil feeding on phloem chemical contents

To visualize effects of MeJA treatment and feeding damage on the phloem samples' chemical profiles, Principal Component Analysis (PCA) was applied. The score plot acquired clearly shows that MeJA treatment changed the seedlings' chemical contents, as it influenced samples' positions along both the first and second axis, which nearly completely separates MeJA-treated and control seedlings that weevils had not fed upon (Fig. 2A). The loading plot indicates that the chemicals that most strongly influenced positions of these samples were the aromatic alcohols 2-phenylethanol, creosols and cymen-8-ol, but also several monoterpenes such as the main constituent (+)-3-carene (Fig. 2B).

Changes in the MeJA-treated seedlings' main monoterpene contents were detected, notably they contained less (+)-3-carene and (+)-α-pinene than the controls (Fig. 3), but tended to contain more (−)-β-pinene ($F_{1,28} = 2.77$, $P = 0.11$). MeJA-treated seedlings also had lower phloem contents of total mono- ($F_{1,28} = 20.83$, $P < 0.001$) and sesqui-terpenes ($F_{1,28} = 6.41$, $P = 0.02$) than controls. However, MeJA treatment increased both phloem and needle contents of the aromatic alcohol 2-phenylethanol (Fig. 4).

The PCA score plot obtained also demonstrates that pine weevil feeding influenced the chemical contents of the phloem samples, which particularly shifted their positions on the second axis (Fig. 2A). These changes included increases in the phloem contents of several monoterpenes, including (+)-α-pinene (Fig. 3), (−)-β-pinene (Fig. 3) and (−)-α-pinene ($F_{1,28} = 5.11$, $P = 0.03$; App. A Table S1). Notably, following weevil feeding stronger increases in phloem levels of the pine weevil attractant (+)-α-pinene were detected in controls than in the MeJA-treated seedlings (*t*-test; $N = 16$; $P = 0.04$).

2.2. Effects of MeJA treatment on weevil feeding behavior

In the parallel no-choice behavioral experiment (Fig. 1), analysis

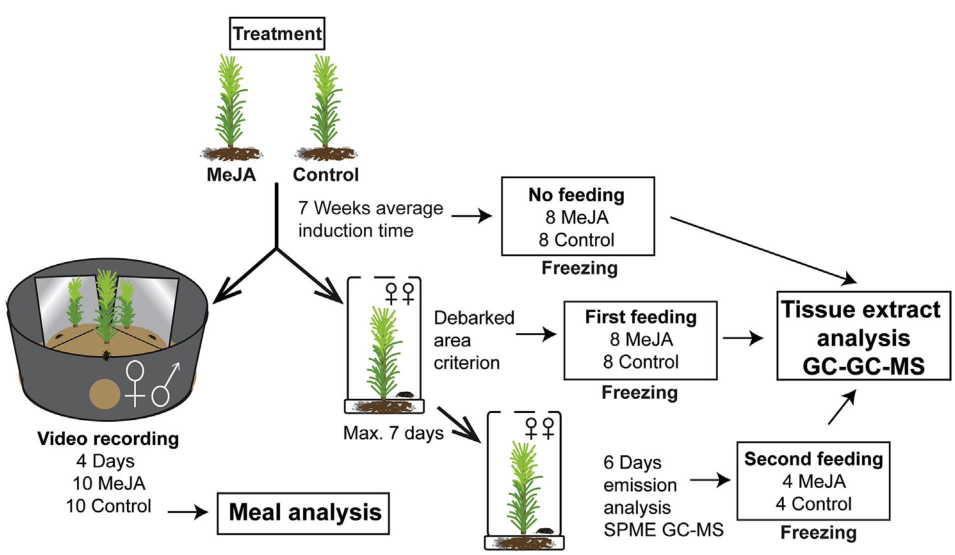

Fig. 1. Overview of the experiments. All MeJA-treated and control seedlings were treated together and assigned at the start of the experiments to the parallel behavioral and chemical experiments. In the behavioral experiment one seedling of each treatment was video-recorded for 4 days with a female or male pine weevil ($N = 5$ seedlings per treatment and sex). In the chemical experiments MeJA-treated and control seedlings were exposed to consecutive feeding sessions followed by destructive sampling of phloem and needles with solvent extraction for GC-MS analysis (for the consecutive step sets of $N = 8$, $N = 8$ and $N = 4$ seedlings were used per treatment). A subset of seedlings ($N = 4$ per treatment) were sampled and analyzed for emissions by SPME GC-MS.

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