Essential Oils as Allelopathic Agents: Bioconversion of Monoterpenes by Germinating Wheat Seeds

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Keywords: Monoterpene, carvone, metabolism, detoxification

Abstract

Monoterpenes present in essential oils are very powerful germination inhibitors. In this study we describe the fate of a number of components of essential oils, after they are applied to wheat seeds to inhibit seed germination. Wheat seeds exposed to exogenous application of essential oils are able to metabolize their monoterpene components.

A high correlation between the level of inhibition of seed germination and the amount of total metabolites in the seed was observed. This suggests that the catabolism of monoterpenes is part of an existing detoxification process. Most of the monoterpenes were either reduced or oxygenated. The products and possible metabolic pathways of various monoterpenes such as carvone, α-thujone, artemisia ketone, α-terpineol, γ-terpinene, p-cymene and δ-3-carene are discussed. Our findings demonstrate the potential of essential oils as allelopathic agents and provide an insight into their mode of action. This information can also be used to develop new methods for converting essential oils to new natural chemicals.

INTRODUCTION

Evidence for allelopathic interactions in nature caused by aromatic plants containing volatile allelochemicals have been described frequently (Muller et al. 1964, Rice 1984). Essential oils were reported as inhibitors of seed germination and plant growth (Asplund, 1968). We have previously reported on the effect of monoterpenes from a number of aromatic plants on the germination of wheat (Dudai et al. 1993, 1999) and have also shown that wheat seeds exposed to defined monoterpenes, such as citral, citronellal, pulegone and and carvacrol are able to metabolize it (Dudai et al. 2000).

The fate of exogenous monoterpenes applied to germinating seeds has not been studied previously. In this study, we describe the fate of a number of components of essential oils, when they are applied to wheat seeds as inhibitors of germination.

MATERIALS AND METHODS

Seed Germination

Wheat seeds, *Triticum aestivum* L. " Dariel" were germinated in glass vials, 25 ml, on three layers of filter paper, (Whatman No. 1) wetted with 1.5 ml distilled water. Vials containing 20 seeds were incubated at 27 ^{o}C in the dark. To test the inhibitory effect of the essential oil components, a known amount of every examined monoterpne was loaded (using a calibrated glass microcapillary) on a piece of filter paper, which was attached to the inner side of the cover of the vial. The vials were closed hermetically. Amounts of up to 2 μl of oil were applied in this way. The oil per vial (1 μl) was equivalent to 40 nl/ml. Experiments were repeated in five replicates. After 24 h seeds were extracted with methyl *tert*-butyl ether (MTBE). The examined monoterpenes were: carvone, (E)-carveol, (Z)-carveol, pulegone, artemisia ketone, linalool, α-terpineol, γ-terpinene , p-cymene and delta-3-carene.

Determination of Essential Oils in the Seed

The seeds were washed by mild shaking for five sec in distilled water and immediately extracted with MTBE, containing 10 μg/ml iso-butylbenzene as an internal

Proc. XXVI IHC – Future for Medicinal and Aromatic Plants
Eds. L.E. Craker et al.
Acta Hort. 629, ISHS 2004
Publication supported by Can. Int. Dev. Agency (CIDA)

standard, for 24 h with gentle shaking at room temperature. The samples were analysed using an HP-GCD apparatus equipped with a HP-5 MS (30 m x 0.25 mm) fused silica capillary column. Helium was used as the a carrier gas. Injection temperature was 250 ^{o}C, the transfer line temperature was 280 ^{o}C. Column conditions were: 70 ^{o}C for 2 min, followed by 4 ^{o}C/min to 200 ^{o}C. The components were identified by co-injection with authentic samples and by comparison of the EI-MS obtained from computerised libraries.

RESULTS AND DISCUSSION

Seeds were exposed to various monoterpenes in the gaseous phase. The content of each applied compound and its derivatives determined in the endosperm and embryo using GC-MS analysis. These compounds were detected both in the endosperm and the embryo. The derivatives did not appear when heat killed seeds were used indicating that the formation of these substances is due to the biological activity of the seeds and not spontaneous. The monoterpenes and their metabolites accumulated during the first day of inhibition are shown in Fig. 1. The amount of monoterpene either remained constant or decreased. There is high correlation between the inhibition of germination of the compounds and the amount of total metabolites determined in the seed (Fig. 1). This suggests that the catabolism of monoterpene is part of a detoxification process, and that the degree of inhibition is related to the ability of the seed to metabolize the monoterpene. The presence of low amounts of monoterpenes may be due to higher rates of metabolism and not the result of lower uptake.

The products and possible metabolism pathways of various monoterpenes are shown in Fig. 2. The ketone monoterpene carvone was reduced to (E)+(Z)-dihydro-carvone and (E) + (Z)-carveol. (E)-dihydrocarvone was reduced to neo-dihydrocarveol and dihydrocarveol (Fig. 2). Similary the ketonic monoterpene pulegone was converted to isopulegol and menthone (Dudai et al., 2000). This suggests the existence of a non-specific mechanism, which can result in the reduction of ketones. In the same way, artemisia ketone was reduced to artemisia alcohol (Fig. 2). The monoterpene alcohol carveol was reduced to dihydrocarveol, which further was reduced to carvomenthol or oxygenated to carvenone. Oxygenation also occurred in the cases of linalool (to 8-acetoxylinalool), α-terpineol (to sobrerol) and the hydrocarbone terpenes γ-terpinene, p-cymene , and δ-3-carene.

The number of quite different substances, which are either reduced or oxidized, might indicate that non-specific enzyme systems are involved. The degradation of essential oils requires the activity of several enzyme systems. Thus, reductive processes might be catalyzed by non-specific dehydrogenases (Plapp et al., 1993) and oxidation might be due to the presence of cytochrome P-450 type enzymes, which today are known to be present in plant tissues and are involved in the biosynthesis of terpenes (Mihaliak et al., 1993; Halkier, 1996). Our findings demonstrate the potential of essential oils as allelopathic agent and provide an insight into their mode of action. This information can be used to develop new methods for converting essential oils to new natural chemicals.

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Figures

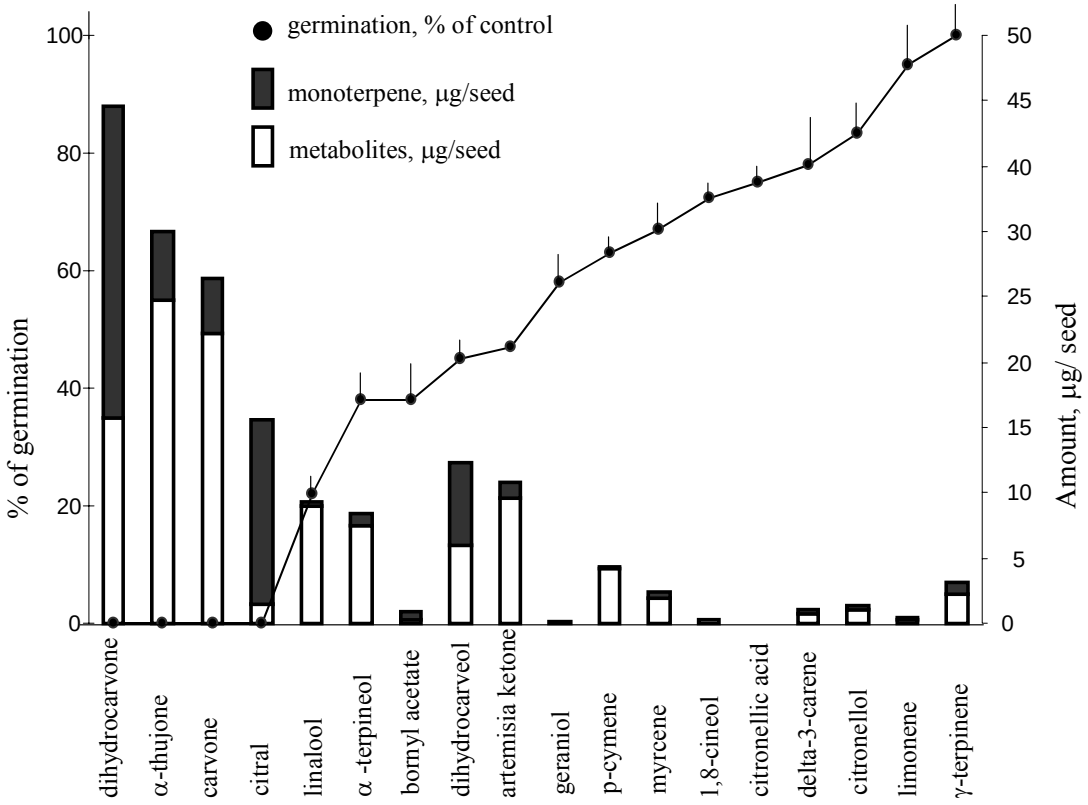

Fig. 1. The monoterpenes and their metabolites accumulated during the first day of inhibition.

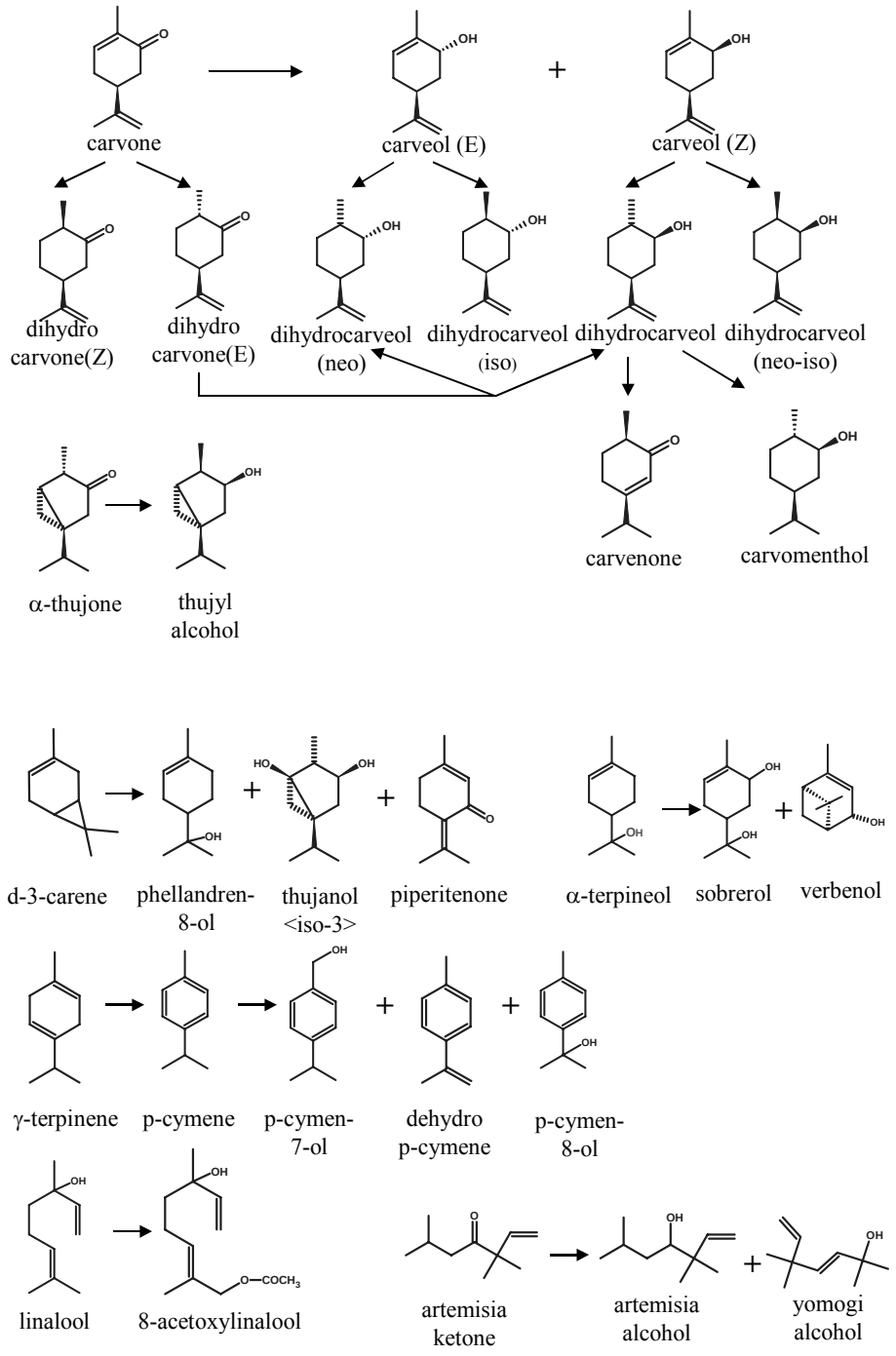

Fig. 2. Products and possible metabolic pathways of various monoterpenes in seeds.