mained stable and were similar to those found in the nonfumigated species, 93.5 % and 6.5 % for (−)- and (+)-α-pinene, respectively (Table 2). Moreover, in all the analyzed samples the total amount of the monoterpenes as well as the percentual distribution of the individual monoterpenes were similar.

However, it should be left open whether the ratio of (−)/(+)-α-pinene remains unchanged after treatment of the trees at elevated O_3 and SO_2 concentrations (≧ 100 ppb) or after other stress conditions, e.g., water stress.

Similar experiments are being planned on selected shrubs belonging to the Mediterranean macchia, e.g., rosmarine, lavender, salvia, etc. It can be anticipated that as far as the distribution of the optical isomers is concerned, the ratio of (−)-α-pinene/(+)-α-pinene in rosmarine, salvia, and lavender compared to that of the investigated spruce trees was quite different, i.e., 0.8, 2.0, and 0.8, respectively.

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1. Schindler, T., Kotzias, D.: Naturwissenschaften *76,* 475 (1989); Kotzias, D., Fytianos, K., Geiss, F.: Atmos. Environ. *24A,* 2127 (1990); Stangl, K., Kotzias, D., Geiss, F.: Naturwissenschaften *75,* 42 (1988)
2. Banthorpe, D. V., Charlwood B. V., Franics, J. O.: Chem. Rev. *72,* 115 (1972); Tavarin, E., Fields, W., Cobb, I., Bergot, J., Barber, H. W.: Phytochemistry *10,* 3107 (1971); Hanover, W.: Mitt. Forstl. Bundesversuchsanst. Wien *97,* 625 (1972); v. Rudlof, E., Lapp, M. S., McMinn, R. G.: Can. J. For. Res. *15,* 801 (1985); Schönwitz, R., Mercck, L., Ziegler, H.: Trees *1,* 88 (1987); Merck, L., Kloos, M., Schönwitz, R., Ziegler, H.: ibid. *2,* 45 (1988); Jüttner, F.: Physiol. Plant. *72,* 48 (1988); Bufler, U., Seufert, G., Jüttner, F.: Environ. Pollut. *68,* 367 (1990)
3. Lindström, M., Norin, T., Birgersson, G., Schlyter, F.: J. Chem. Ecol. *15,* 541 (1989)
4. Seufert, G., Staudt, M., Kotzias, D., Frenzel, B.: Int. Conf. on Acidic Deposition, Glasgow 1990. The Balance of Gas Exchange (SO_2, O_3, CO_2, H_2O, biogenic hydrocarbons) in Continuous Stirred Tree Exposure Systems

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Mineralization Kinetics and Utilization as an N Source of Dicyandiamide (DCD) in Soil

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Dicyandiamide (DCD, cyanoguanidine) is a widely used nitrification inhibitor in German agriculture. The inhibitory effect of DCD, however, varies considerably and depends upon its stability in soil. In early investigations [1, 2], mainly physicochemical mechanisms affecting dicyandiamide decomposition were considered. Recently, several bacteria degrading DCD in pure cultures were isolated [3 – 5] confirming the microbiological rather than chemical nature of DCD decomposition in soils. If DCD is metabolized by a specific part of the soil microflora, DCD decomposition should show a clear lag phase (time required to induce the enzymes involved) and temperature dependence. DCD contains ca. 66 % N (w/w) and the question arises whether microorganisms may use this compound as a single nitrogen source.

Two sets of incubation experiments (each in three replicates) were carried out using Didin*-pretreated (30 kg ha^{-1} three times a year) or non-pretreated grassland silt loam soil (pH 6.1, C_t 2.9 %, N_t 0.25 %) [6]. Five-hundred-g portions of the pretreated or non-pretreated air-dried soil (< 2 mm) were carefully mixed with dicyandiamide (16.7 μg DCD-N g^{-1} dry soil), moistened (80 % WHC), incubated (10, 20, and 30 °C), and analyzed for DCD [7] at regular intervals. In the second set of experiments, the effect of a simple carbon source (citrate) on the utilization of DCD by soil microorganisms was examined. A neutral phosphate buffer solution of Sörensen (165 ml distilled water containing 0.885 g KH_2PO_4 and 1.81 g $Na_2HPO_4 \cdot 2H_2O$) was autoclaved (15 min at 121 °C) in 1-l Erlenmeyer flasks together with 10 ml of trace elements solution (Hoagland). After cooling the sterilized solutions (each 175 ml) to room temperature, 25 ml of sterile filtered (membrane filter 0.2 μm, Schleicher & Schuell, Germany) dicyandiamide-N (25 mg) and sodium citrate (0.405 g) solution were added to each flask. The flasks were finally inoculated with 50 ml of a soil extract prepared by shaking (1 h) 500 g of air-dried non-pretreated soil with 0.5 l sterile distilled water (with 0.18 % $Na_4P_2O_7 \cdot 10H_2O$) and subsequent filtering. The flasks were continuously shaken (25 °C in darkness) over an orbital shaker at 100 rpm. Dicyandiamide [7] and citrate [8] contents were measured quantitatively at regular intervals using a Hitachi U-3200 spectrophotometer.

In Fig. 1 the decrease in DCD in the pretreated (a) and non-pretreated (b) soils at different temperatures is compared. In the pretreated soil mineralization started immediately at all temperatures (10, 20, and 30 °C), while in the non-pretreated samples a clear lag phase was recorded (at 10 and 20 °C, respectively). The lag phase shortened with increasing temperature, suggesting a rapid enzymatic adaptation of a part of the microflora. At 30 °C microbial adaptation is rapid and DCD mineralization started nearly without delay even in the non-pretreated soil. Once adapted, microorganisms decompose DCD at a constant rate following zero-order kinetics. Microbial adaptation and temperature are two important

* Commercial name of dicyandiamide produced by SKW AG, Trostberg, Germany

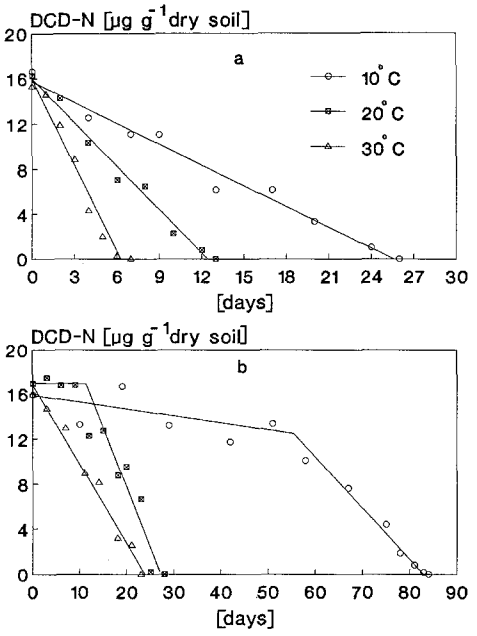

Fig. 1. Decomposition kinetics of dicyandiamide in pretreated (a) and non-pretreated (b) soil at 10, 20, and 30 °C, respectively. Note the different scales on the axes

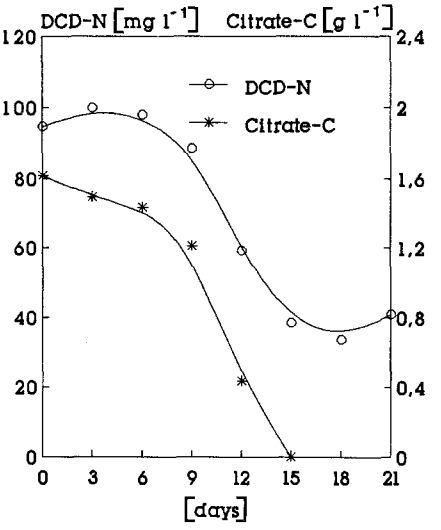

Fig. 2. Utilization of dicyandiamide in relation to citrate-C consumption by a mixed consortium of soil microorganisms

factors that determine the persistence of chemicals in the soil. Note that DCD disappeared completely in the pretreated soil within 6 – 7 days (30 °C) but could be measured under the same conditions in the non-pretreated soil for up to 24 days. In the pretreated soil the decomposition rate was reduced by about 50 % with every 10 °C decrease. This is characteristic for enzymatic processes. In Fig. 2 the mineralization of DCD by a consortium of soil bacteria in relation to the utilization of citrate is presented. After a short adaptation phase, DCD decomposition proceeded rapidly in the presence of citrate and ceased as soon as the carbon source became exhausted. Apparently, DCD is used as a nitrogen source most probably because of its narrow C/N ratio (ca. 0.4). A complete degradation of DCD (Fig. 2) even after the complete utilization of citrate could be expected, but was not observed during 6 days after the disappearance of citrate.

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1. Amberger, A., Vilsmeier, K.: Z. Pflanzenernaehr. Bodenkd. *142,* 778 (1979)
2. Vilsmeier, K.: ibid. *143,* 113 (1980)
3. Hauser, M., Haselwandter, K.: Soil Biol. Biochem. *22,* 113 (1990)
4. Hallinger, S., Wallnöfer, P. R., Goldbach, H., Amberger, A.: Naturwissenschaften *77,* 332 (1990)
5. Schwarzter, Ch., Haselwandter, K.: Soil Biol. Biochem. *23,* 309 (1991)
6. Kapp, M., Schwarz, J., Benckiser, G., Ottow, J. C. G., Daniel, P., Opitz von Boberfeld, W.: Forum Städte Hyg. *41,* 168 (1990)
7. Vilsmeier, K.: Z. Pflanzenernaehr. Bodenkd. *142,* 792 (1979)
8. White, J. C. D., Davies, D. T.: J. Dairy Res. *30,* 171 (1963)

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3,7,7-Trimethyl-1,3,5-cycloheptatriene in Volatiles of Female Mountain Pine Beetles, *Dendroctonus ponderosae*

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Recently, we have reported that female mountain pine beetles, *D. ponderosae* Hopkins (Coleoptera: Scolytidae), produce monoterpene hydrocarbons such as 4-methylene-6,6-dimethylbicyclohept-2-ene (verbenene), *p*-mentha-1,5,8-triene, and *o*-mentha-1,4,6-triene [1]. Following the discovery that verbenene is a new aggregation pheromone of the spruce beetle, *D. rufipennis* (Kirby) [2], we reanalyzed volatiles of mountain pine beetles in search of monoterpene hydrocarbons which may serve as aggregation pheromones.

Mountain pine beetles emerging from naturally infested lodgepole pine logs, *Pinus contorta* var. *latifolia* Engelmann, were collected, sexed, and 300 – 350 males and females, respectively, inserted into a Pyrex glass tubing (Fig. 1). The aeration device was similar to Rudinsky's "apparatus for collecting volatiles from living beetles in simulated galleries" [3], but beetles were not individually caged within the tube. A constant aspirator-driven and charcoal-filtered airstream was maintained through the tube for 10 days, collecting beetle-released volatiles on Porapak Q (Fig. 1).

GC-MS analyses of volatiles released by emergent, unfed male *D. ponderosae* disclosed the aggregation pheromone *exo*-brevicomin [4, 5], *endo*-brevicomin, and frontalin [6] in a 10.6:1.0:0.01 ratio (Fig. 2). Mass spectra of further unidentified compounds resembled those of bicyclic ke-