EFFECTS OF ALLELOCHEMICALS ON PLANT RESPIRATION AND OXYGEN ISOTOPE FRACTIONATION BY THE ALTERNATIVE OXIDASE

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(Received April 3 1995; accepted: December 5 1995)

Abstract—The goal of this investigation was to determine the effects of allelochemicals on plant respiration that thereby may be responsible for their role in growth inhibition. We have tested the effects of juglone, quercetin, cinnamic acid, and α-pinene on respiration rates, and electron partitioning through the cytochrome and alternative respiratory pathways, by measuring on-line oxygen consumption and oxygen isotope fractionation in soybean cotyledon tissue. Cinnamic acid and α-pinene decreased the oxygen consumption rate and increased the relative partitioning of electron transport to the alternative pathway. Possible biochemical mechanisms of these effects are discussed.

Key Words—Allelopathy, alternative oxidase, α-pinene, cinnamic acid, juglone, on-line ¹⁸O fractionation, quercetin, respiration, soybean.

INTRODUCTION

There remains considerable controversy as to the ecological relevance of allelopathy, and not all ecologists accept the concept as a significant competitive factor in plant communities (Harper, 1977; Harborne, 1993; Peñuelas, 1993).

We aimed to determine the possible effects of allelochemicals on plant energy metabolism and consequently their role in growth inhibition. We have tested the effect of some typical, widely occurring allelochemicals from different

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chemical families (the quinone juglone, the flavonoid quercetin, the phenolic cinnamic acid, and the terpenoid α-pinene) on soybean cotyledon respiration rates and on partitioning through cytochrome and alternative respiratory pathways. We studied the respiratory response of soybean cotyledons, these being one of the most widely used vegetable materials in plant respiration studies. We avoided problems associated with the traditional use of inhibitors such as SHAM or KCN by using on-line fractionation against 18O, which is substantially greater by the alternative oxidase than by cytochrome oxidase (Guy et al., 1989; Robinson et al., 1992).

METHODS AND MATERIALS

We conducted on-line measurements of oxygen consumption and oxygen isotope fractionation (Δ) during dark respiration by soybean cotyledons. Immediately before recording the measurements, 30 soybean cotyledons in darkness were bathed for 30 min in CaCl2 aqueous solutions with 10 mM of each of the tested allelochemicals (quercetin, juglone, and cinnamic acid from Sigma Chemical Co.) except for α-pinene (also from Sigma Chemical Co.), which was kept in an open beaker volatilizing inside the gas-tight syringe chamber we used to measure respiration.

Full details of the equipment and methods for these on-line measurements are described in Robinson et al. (1992). In these experiments we introduced the following modifications and improvements. Thirty soybean cotyledons inside a gas-tight 50-ml chamber were allowed to respire. At regular time intervals, gas samples of 100 µl were withdrawn and injected directly into helium flow to a GC-MS, after stripping water and CO2. For all the measurements the room temperature was 25°C. The fractionation factor (D) was calculated as the slope of the linear regression of ln(R/R0) versus −ln f through the origin (Guy et al., 1989), where f is the fraction of the original O2 in the reaction chamber, and R0 and R are the isotope ratios (18O/16O) of the initial and subsequent samples (for all the measured relationships r² was larger than 0.99). The fractionation Δ was calculated from D using the equation Δ = D/(1 − D). These experiments consisted of 8–12 oxygen measurements and were replicated three to six times for each of the allelochemicals studied.

RESULTS AND DISCUSSION

Cinnamic acid and α-pinene (the smaller molecules tested, and therefore the ones more likely to penetrate live tissues) decreased the oxygen consumption rate, while quercetin and juglone had no significant effect (Table 1). These results agree with data suggesting perturbations of mitochondrial and chloroplast
functions linked to plant growth reductions observed in classical allelopathy experiments (Pellessier, 1993; Hejl et al., 1993; see references in Einhellig, 1995). However, they do not agree with the general hierarchy of allelopathic activity: quinones > flavonoids > phenolic acids (Einhellig, 1995).

The fractionation (Δ) ranged between 31% when KCN was used to inhibit the cytochrome pathway and 20% when SHAM was used to inhibit alternative oxidase. The Δ of 24.3% in control treatments was increased to 26.8% by cinnamic acid treatment (Figure 1), indicating an increased percentage of respiratory flux to the alternative pathway from 39 to 62%. The relative partitioning of electron transport to the alternative oxidase pathway was also increased (higher Δ) by α-pinene (Table 1), but was not significantly changed by quercetin or juglone (Table 1). However, none of these allelochemicals changed the absolute electron flux through the alternative pathway (Table 1). Thus, the inhibition of respiration occurred in the cytochrome pathway and possibly in glycolysis; otherwise the redox state of the ubiquinone pool would have increased and enhanced the flux through alternative pathway. The effects on respiration could be secondary through other cell functions, such as, for example, ion uptake, which are energy demanding. Further work on isolated mitochondria is necessary to clarify the situation.

The studies conducted here dealt with responses of isolated allelochemicals,
and in the short-term, of the order of 1 hr. Of course, such an approach is far from the extreme chemical complexity of the soil and air environment, and additional studies are required to determine how and at what concentrations these substances become actual allelochemicals. Nevertheless, the method used here provides an indication that these allelochemicals alter normal oxygen uptake and may influence significant competitive effects on neighboring plants.

Acknowledgments—We thank Dr. J. Siedow for his help with the revision of the manuscript. The financial support of NSF DEB#9112571 to the Duke University Phytotron, and of CICYT-AMB94-0199 and INIA-SC94-011 (Spain) to J. P. is gratefully acknowledged. M. R. C. received a MEC-Fulbright Postdoctoral Fellowship during the course of the work and was funded by USDA NRI-CGP94-37306-0352.
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