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BIOGENESIS OF CARBON MONOXIDE: PRODUCTION BY FUNGI AND SEED PLANTS IN THE DARK

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Key Word Index—Fungi; angiosperms; carbon monoxide; dark production.

Abstract—Fungi and angiosperm seeds and seedlings produce carbon monoxide in darkness when under a low oxygen–high carbon dioxide atmosphere. Peak yields of 40 to 90 nmol/g dry weight were measured after six days, declining thereafter. Peak rates of carbon monoxide (CO) production were observed in *Agaricus* and cucumber seedlings (0.65 and 0.91 nmol/g/hr, respectively). The addition to radish and cucumber seeds of an antibiotic mixture had little effect on the course of their CO production. We suggest that in addition to the well known process of CO production in leaves of terrestrial plants in light, there is a significant light independent source of the gas among smaller plants associated with the soil–surface and soil–air interface.

INTRODUCTION

Since Langdon's original report on the occurrence of free carbon monoxide (CO) in the kelp *Nereocystis* in 1916 [1], the presence in and biogenesis of the gas have been the subject of a number of publications. Coelenterate animals and fungi, as well as higher green plants have been found to be active CO producers [2–8]. The magnitude of biogenic CO release into the environment has been remarked upon by Delwiche [2] and a decade ago Seiler and his associates at the Max Planck Institute for Chemistry (Mainz) concluded that "... plants may contribute significantly to the atmospheric CO-cycle with production rates comparable to the total CO-production in the ocean" [9, 10]. These authors also emphasize the light-dependent character of CO production by terrestrial organisms at substantial fractions of solar radiation intensity, but by conducting their measurements at atmospheric levels of oxygen (*ca* 20%) they may have overlooked alternative plant sources of CO, namely seeds, seedlings and fungi which are found in the soil microenvironment. In this important niche, seeds, seedlings and microbiota constantly modify the atmosphere through metabolic processes. Thus, the upper horizons of agricultural soils in a normally wet condition may contain elevated carbon dioxide with oxygen at 10% (v/v) or less [11–15]. In paddies, bogs or other wetlands environments, there may be no molecular oxygen at all.

In 1962, we described experiments in which plants, seeds and seedlings in darkness produced CO in atmospheres containing 5% oxygen or less and elevated carbon dioxide levels [16]. Thus in view of the importance that has since been placed upon the role of plants in the regulation of atmospheric gases, we have now further examined plants as candidates for dark biogenesis of CO.

During an initial three days of uninterrupted darkness, *Agaricus* yielded nearly 70 nmol/g and *Auricularia ca* 30 nmol/g of CO (Fig. 1). Gas sampling, required only five min in dim room light, and was followed by three additional days of dark production to a six-day maximum of 95 and 50 nmol/g, respectively. During these initial samplings, the gas retained the usual 'mushroom' odour with no signs of microbial contamination. Subsequently, when bacterial growth was evident between sample days 12 and 30, and gas samples acquired an odour of putrefaction, CO levels were in rapid decline.

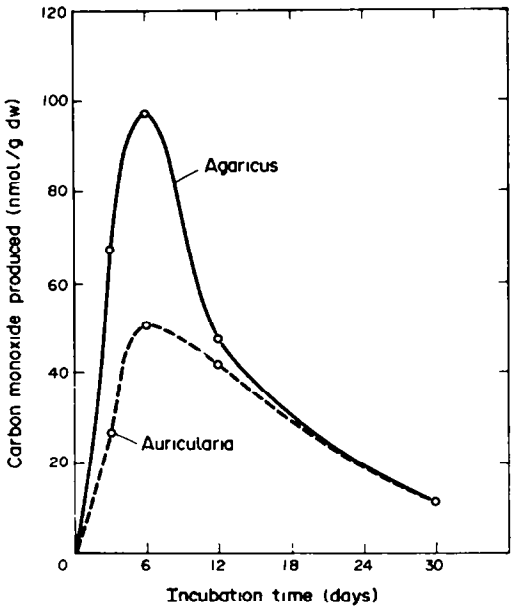

Fig. 1. Time course of CO production by fruiting bodies of the Basidiomycetes *Agaricus campestris* and *Auricularia auricula*. Tissues were incubated in nitrogen containing 5% oxygen in darkness at 24°.

The dark production of CO by the fungus *Aspergillus* was reported by Westlake *et al.* in 1961 [7] but only from

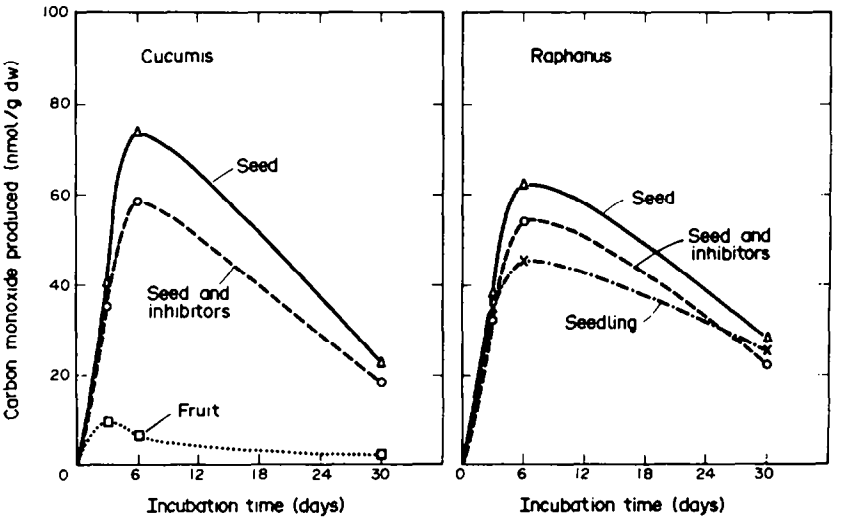

Fig. 2. Time course of CO production by green plants in darkness at 24°. Left: Cucumber (*Cucumis*) seeds in water or microbial inhibitor solution, and cucumber fruit. Right: Radish (*Raphanus*) seeds in water or microbial inhibitor (250 000 units of penicillin V potassium: 200 000 units mycostatin; 2 g streptomycin; 10 g mixed sulphonamides per litre) solution, and radish seedlings.

the degradation of flavonoids hydroxylated at carbon atom 3. This may be of some importance in those instances where decaying bryophytes or vascular plants are present to provide the flavonoid source.

The yield of CO from cucumber and radish seed followed a course similar to those described by the fungi (Fig. 2). It is not certain, however, that the peak output was near day 6, because no 12-day sample was taken. Nevertheless, the pattern seems similar, including a high three-day output under continuous darkness. Again, as we noted with the fungi, the plant material appeared to be uncontaminated by bacteria at the time of three- and six-day samples. This was most positively indicated by the clarity of the aqueous phase added to the seeds before their flasks were sealed. As an extra precaution, however, a set of vessels were prepared differing from the above only in the aqueous inhibitor solution used in place of water (Fig. 2).

Whether or not the slightly reduced yield of CO was the result of antimicrobial action or phytotoxicity is not known, but it shows clearly that the plant material is the major producer of the gas. By day 30, the aqueous medium supplied seeds without inhibitor was quite turbid whereas the antimicrobial medium was entirely clear.

Production of CO by etiolated, 10-day-old radish seedlings resembled their seeds, but cucumber fruit CO output was miniscule compared with cucumber seed. Even after 30 days, when the fruit was visibly contaminated with bacterial and fungal growth, CO output remained low. During the first three days incubation, CO_2 rose from its initial level of < 100 ppm by volume (ppmV) to about 1% v/v, and continued to rise thereafter. For the comparison of production rates, an incubation period of six days was taken to be at or reasonably close to the time of maximum output (Table 1). At this time, autoclaved seeds produced essentially no CO.

With the exception of the extreme low value found for cucumber fruit, the range for all species and stages was 0.23 to 0.92 nmol/g/hr, which compares well with our previous 5-day measurements of 0.11–0.56 nmol/g/hr [5]. In two instances, with cucumber fruit and radish seedlings, exposure continuously to a 40 W 'daylight' fluorescent light source at 2 m distance had no influence on CO production.

Table 1. Carbon monoxide emission by fungi and seed plants compared after six days incubation at 24° in darkness under a 5% O_2 atmosphere. Rates are averages of duplicate determination or means of triplicates with standard deviations.

Species	CO emission rate nmol/g/hr
Fungi	
Agaricus campestris	0.65 ± 0.08
Auricularia auricula	0.32 ± 0.08
Seed Plants	
Cucumis sativus	
seed	0.51 ± 0.12 (0.29)
autoclaved seed	< 0.05
seedling	0.91 ± 0.16
fruit	0.05
fruit + light	0.04
Raphanus sativus	
seed	0.41
autoclaved seed	< 0.05
seedling	0.29 ± 0.05
seedling + light	0.28 ± 0.06
Phaseolus aureus	0.23
Brassica oleracea var Capitata	0.28

At high light intensities, Fischer and Lüttge [18] found that shoots of oleander (*Nerium*) produced CO at about 0.8 nmol/g/hr when the atmosphere contained about 5% O_2 and 275 ppmV CO_2, and at about 0.6 nmol/g/h when the atmosphere contained 21% O_2 and 300–2200 ppmV CO_2. They did not test the equivalent of our low-oxygen-rising carbon dioxide condition, and reported an "in-

significant" release of CO in air and darkness, approximately equivalent to our low output figure for cucumber fruit.

Our experiments suggest that some plants, both saprophytic and photoautotrophic contain a non-light requiring pathway for CO production which is favoured by the conditions found on and within upper soil layers, and of sufficient magnitude to contribute to the overall CO flux, especially at night when the better known photoprocess is inoperative, or in dense forest where photoprocesses are energy limited.

EXPERIMENTAL

In our original study, the results of measurement using colour changes in Mine Safety Appliance detector tubes were in satisfactory agreement with those obtained by gas chromatography in the working range of 2.5–1000 ppmV [16]. In this report, the data were obtained using the Dräger colorimetric tube procedure with a working range of 2–300 ppmV [19].

Compared with the methods described by Seiler and others, the Dräger tubes are less sensitive and somewhat more variable as a result of reading and interpolation errors. Nevertheless, our experience has shown that 2–10 ppmV can be reproduced to $\pm 15\%$ and higher concentrations even more closely. The method is rapid, simple and requires only 100–300 cm^3 sample volumes under our experimental conditions. Experimental vessels were 1.2–3.5 l, and the seed, plant or fungal biomass introduced was equivalent to 35–100 g of dry matter. The biological test materials included fruiting bodies of the edible fungi *Agaricus campestris* and *Auricularia auricula*, commerical cucumber (*Cucumis sativus*) and egg plant (*Solanum melongena*) fruit, and seeds or seedlings of cultivated plants including *Brassica oleracea* var. *capitata*, cabbage; *Cucumis sativus*, cucumber; *Glycine max*, soybean; *Phaseolus aureus*, mung bean, and *Raphanus sativus*, radish. All plant materials were weighed and washed in distilled H_2O just before being placed in their flasks. Percentage dry wts were determined on separate portions of the plant tissue. Before loading, flasks were shaken several times with 0.1% NaOCl solution and allowed to drain without rinsing.

Tissues were allowed to incubate in 5% O_2 atmospheres and sampled at intervals for up to 30 days. Sample tube readouts in ppmV were converted to nmol/l [6]; CO production calculated as nmol/g, and rates as nmol/g/hr. All values are based on duplicate or triplicate samples, the latter given with standard deviations.

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