Basil Chlorosis: A Physiological Disorder in CO_2-Enriched Atmospheres

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ABSTRACT

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Basil (*Ocimum basilicum*) grown commercially in a hydroponic facility under high-pressure sodium lights in a CO_2-enriched atmosphere developed a distinct interveinal chlorosis. Attempts to transmit the disease mechanically or by grafting to unaffected, greenhouse-grown basil and certain other plant species failed. Affected basil that was transferred from the hydroponic facility to a greenhouse and grown in potting mix produced new, chlorosis-free leaves. Under experimental conditions, basil became chlorotic in 1,000 ppm CO_2, but not when in ambient CO_2. This was true whether the basil was grown hydroponically or in pots, or under fluorescent or high-pressure sodium lights. Electron microscopy revealed no detectable pathogens, but large starch grains were observed in chloroplasts of chlorotic leaves. Elevated CO_2 concentrations apparently induced basil chlorosis.

Basil (*Ocimum basilicum* L.) is a seasoning herb grown commercially throughout the year both in soil in greenhouses with natural light and ambient CO_2 and in hydroponic facilities where artificial lights are the only light source and the atmosphere is enriched with CO_2. Hydroponic basil may produce significantly more fresh weight and essential oils than soil-grown plants (5). During 1986 and 1987, a distinct interveinal chlorosis of basil grown under artificial light in a CO_2-enriched atmosphere was observed at two different hydroponic facilities, including Phytofarms of America in DeKalb, Illinois. Purchasers of the chlorotic basil complained about the yellow leaves, thus reducing its commercial value. Because the chlorosis resembled the mosaic symptoms of certain plant virus infections, we investigated the nature of the disease first by testing for transmissibility and then by attempting to induce the disease under various greenhouse conditions. We present evidence that CO_2 enrichment is the key factor in the induction of this apparently physiological disorder.

MATERIALS AND METHODS

Basil cultivar Crispum for commercial production was grown hydroponically at 24–27 C under either 400W or 1,000W high-pressure sodium (HPS) lights (100–250 $\mu E \cdot m^{-2} \cdot s^{-1}$) in an atmosphere with the CO_2 level maintained at 1,000 ppm as described by Zinnen (10). Basil also was grown experimentally at Phytofarms and at Northern Illinois University at 24–27 C in 20-L aquariums. Plants in the aquariums were supported by perforated Styrofoam panels, and the standard nutrient solution was aerated using an aquarium air pump. Standard nutrient solution (1×) was a modified Hoagland's solution with a conductivity of 2 mmho. The solution was changed weekly.

Tests of transmissibility. Leaves were ground in 30 mM sodium phosphate (pH 8) and juice was wiped on corundum-dusted leaves of Crispum basil, lemon basil (*O. citriodorum* L.), *Nicotiana tabacum* L. 'Xanthi-nc' and 'Havana 38', *N. sylvestris* Speg. & Comes, cowpea (*Vigna unguiculata* (L.) Walp.), and cucumber (*Cucumis sativus* L. 'Lemon'). Plants in the infectivity studies were grown in a glass-glazed greenhouse in 400-ml plastic pots containing Metro Mix soilless potting mix.

To test for graft transmission, scions of affected plants were grafted into the axils of unaffected, greenhouse-grown basil. As a control, scions of greenhouse-grown plants were grafted onto similar stocks. Test plants were observed for 3 wk after inoculation or grafting.

To test for transmission by a root-borne agent, a small (10 cm long, including the root), chlorotic, hydroponically germinated basil plant and a similar greenhouse-grown plant were both placed into a pot filled with Metro Mix soilless potting mix. Controls consisted of similar pots containing two greenhouse-grown plants. Transplants were placed in a greenhouse at ambient CO_2 and observed for 4 wk.

Leaf tissues for examination by electron microscopy were fixed in 2.5% glutaraldehyde, postfixed in 1% OsO_4, dehydrated in a graded ethanol series, embedded in Mollenhauer's resin (7), and stained in uranyl acetate and lead citrate. Sections were viewed in a Hitachi HS-9 transmission electron microscope.

RESULTS

Description of basil chlorosis. Basil seedlings grown hydroponically in 1,000 ppm CO_2 developed interveinal chlorosis within 2 wk of germination; the chlorosis became more pronounced with further growth. The distribution of affected plants was apparently random. The incidence was sporadic in 2-wk-old seedlings but approached 100% at time of harvest (3 wk later). Aphids, whiteflies, leafhoppers, or other common insect vectors of plant viruses were not observed. A waterborne vector or mechanical transmission of an infectious agent occurring at transplanting could not be ruled out. Other taxa of basil, such as lemon basil and holy basil (*O. sanctum* L.), and crops such as lettuce (*Lactuca sativa* L. 'C_{26}') and spinach (*Spinacia oleracea* L. 'Carambole') remained unaffected when grown under conditions identical to those of the affected basil.

Lack of transmission. Because the chlorosis resembled symptoms of certain plant virus infections, we first tested whether the disease was transmissible to greenhouse-grown plants by mechanical inoculation using juice from affected leaves of plants grown in the hydroponic facility. No symptoms were observed on any indicator. No chlorosis developed in any of the five stock plants grafted with chlorotic scions or in the control plants. Chlorosis also failed to develop in any of the 10 indicator plants or corresponding control plants.

Electron microscopy. Because transmissibility assays proved negative, we sought direct evidence of a pathogen by electron microscopy of affected plants. We examined yellow leaf tissue and adjacent green tissue from affected basil grown hydroponically and green leaf tissue from basil grown in soil under fluorescent lights. While no viruslike particles or inclusions were seen, structures identified as starch grains found in chloroplasts from chlorotic tissue were markedly larger than those from adjacent green tissue or from leaves grown under fluorescent light (Fig. 1).

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Experimental induction of chlorosis. Hydroponically grown chlorotic basil was transplanted into potting mix and grown in the greenhouse or in a growth chamber under continuous fluorescent light and ambient air. Such plants produced new leaves without marked chlorosis. Their new leaves were narrower and shorter, especially those on plants grown under fluorescent light. Basil grown from seed in soilless mix in the greenhouse or in the growth chamber also produced green, narrow, and short leaves compared to plants grown commercially under hydroponic conditions.

While not eliminating the possibility of a seedborne pathogen, these observations established that the seed lot could produce healthy plants under greenhouse conditions. When coupled with the apparent nontransmissibility of the disease, these observations led us to test the hypothesis that the chlorosis disease was a physiological disorder.

There were three major differences between the conditions at the hydroponic facility and those in the greenhouse: nutrient solution, type of light, and atmospheric CO_2 concentration. To test whether growth under HPS lights was sufficient to induce chlorosis, basil plants grown in pots containing soilless mix were placed either in the greenhouse under natural light or in a growth chamber under two 400W HPS lights with ambient CO_2. Plants were treated weekly with a 0.5% solution of soluble fertilizer (20-20-20). During 4 wk of culture, we did not observe distinct interveinal chlorosis comparable to that of basil grown hydroponically at Phytofarms. However, plants grown in soilless mix at Phytofarms did develop chlorosis.

To test whether hydroponic culture under HPS light was sufficient to induce chlorosis, basil was grown hydroponically in 20-L aquariums in ambient CO_2 at Northern Illinois University. At the same time, basil was grown experimentally at Phytofarms in a similar manner, except for higher CO_2 concentration. No distinct interveinal chlorosis developed in basil grown hydroponically in ambient CO_2. However, the basil grown at Phytofarms developed distinct interveinal chlorosis.

This result suggests that chlorosis was caused by "excessive" growing conditions. Perhaps the combination of long light periods, high nutrients, and augmented CO_2 levels let photosynthesis occur at such a high rate that the photosynthate produced could not be transported away from the chloroplasts quickly enough. If this was true, reducing the nutrient solution concentration or the light intensity might reduce the incidence of basil chlorosis—an easy method of managing the disease. However, basil grown experimentally at Phytofarms in 0.5× nutrient solution developed chlorosis to the same degree as controls grown in 1× nutrient solution.

DISCUSSION

Although the chlorosis resembles mosaic symptoms observed in certain plant virus infections, we found no evidence for a transmissible pathogen. The disease was observed only in plants grown in a CO_2-enriched atmosphere. Affected plants transferred to or grown in ambient CO_2 produced green, healthy new leaves. While none of these observations preclude the possibility of a pathogen also being involved in the disease, they are all consistent with the hypothesis that basil chlorosis is a physiological disorder induced by a CO_2- enriched atmosphere.

Other workers have reported extensive occurrence of leaf disorders in crops grown in CO_2-enriched atmospheres (6,8,9). For example, the large starch grains we observed in the chloroplasts of chlorotic basil resemble those observed in clover (*Trifolium subterraneum* L.) grown in 1,000 ppm CO_2 (1). Furthermore, bean plants grown in 1,400 ppm CO_2 developed a chlorosis that was amplified by high light intensity (340 $\mu E \cdot m^{-2} \cdot s^{-1}$) or low temperatures (20 C). Starch content of the chlorotic leaves was elevated under either of these conditions (3). In general, previous work has not explored the question of whether the activity of a pathogen can explain such disorders. However, large starch grains also developed in chlorotic lettuce leaves infected by beet western yellow virus (4). In the absence of evidence of a transmissible virus, and in light of the CO_2-enriched atmosphere at Phytofarms, the evidence supports carbon dioxide as the key factor in basil chlorosis.

Because the yellow appearance is unappealing to the consumer, basil chlorosis is of economic importance. Since the disease appears to be of a physiological nature and not caused by a pathogen, its control may be best approached by determining the optimal growth conditions for basil. In ambient CO_2, for example, the fresh weight of basil was greater when grown in 0.5× nutrient solution than in 1× or in 2× solution (*data not shown*). If optimum growing conditions for basil differ significantly from those for spinach and lettuce, as appears to be the case, then means of maintaining two environments in one commercial facility will have to be found. Alternatively, cultivars such as lemon basil that flourish under conditions favorable for spinach can be grown. These cultivars, however, may not be so profitable as the original.

Basil chlorosis is an example of how novel diseases can develop when crops are grown in a greatly altered environment, such as that found in artificially lighted hydroponic facilities (2). The fact that the disease occurs under conditions favorable to lettuce and spinach shows the importance of determining the optimal environmental conditions for each crop cultivated in a facility.

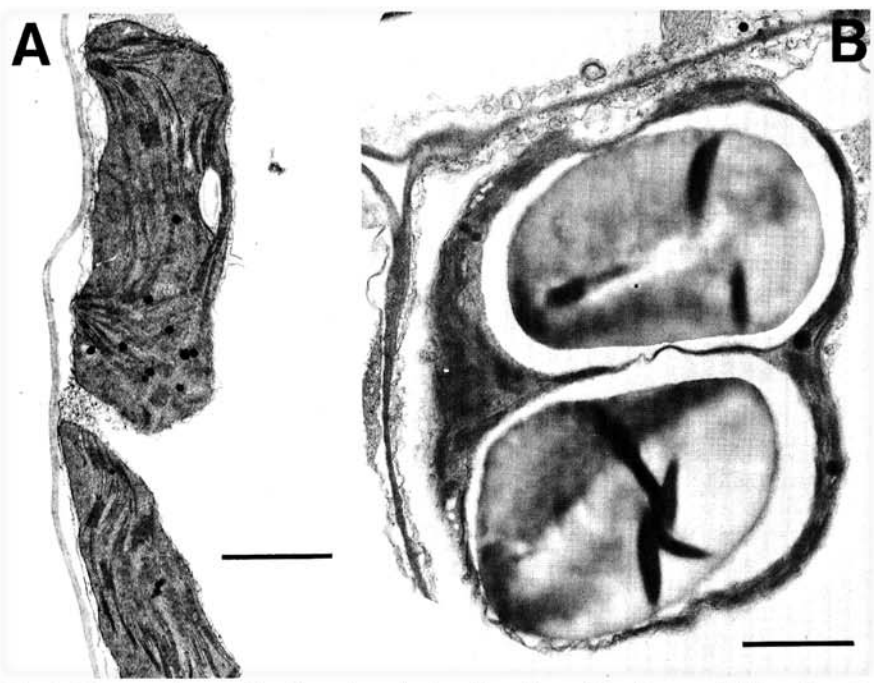

Fig. 1. Electron micrographs of starch grains in chloroplasts of basil grown **(A)** in soil under fluorescent light and ambient carbon dioxide and **(B)** hydroponically under high-pressure sodium light in an atmosphere enriched to 1,000 ppm carbon dioxide. Scale bar = 1 μm.

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