

PROCEEDINGS OF THE "COLD AG" WORKSHOP

An assessment of the use of low cost cold in tropical agriculture

July 28-29, 1992
Kailua-Kona, Hawai'i

Co-Chairs: Dr. John P. Craven and Dr. Ellen Weaver

Editor: Barbara J. Lee

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• Law of the Sea Institute, Richardson School of Law, University of Hawai'i at Manoa
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Cover Photo: It all began with strawberries. These plants were left untended for several months in the early days of the Common Heritage Corp. Demonstration Garden at Keahole Point, and, lo, they tended themselves in the heat of rainless days and bright Kona sunshine, thanks to the cold deep seawater pipes over which they abundantly grew.

TABLE OF CONTENTS

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INTRODUCTION

To Whom It May Concern,

This workshop was convened to assess the use of low cost cold in tropical agriculture, and, by implication, in all phases of aquaculture as well as industrial and commercial cooling processes. The source of this low cost cold is deep ocean water which surrounds our island state in great abundance. The acronym "COLD AG" is employed to identify this new field of research and development.

The "COLD AG" concept was first proposed by the late Dr. Sanford Siegel based on his observation of coldwater condensation resulting from oceanic water flowing through rocks in a Spanish vineyard. He suggested pumping cold deep ocean water through pipes in the ground to induce condensation and create micro-climates favorable for the growing of temperate species in tropical climates. In these speculations the contributions of Dr. Barbara Siegel were integral.

Cold deep sea water was first made available for such use at the Natural Energy Laboratory of Hawaii Authority (NELHA) at Ke-ahole, Hawaii, where it is pumped ashore for use in the OTEC process. OTEC, or ocean thermal energy conversion, exploits the naturally occurring temperature difference between deep and surface waters of the ocean at tropical latitudes to produce energy. Pure seawater enters a series of OTEC heat exchangers at 4° to 6° C, and emerges at 7° to 9° C, otherwise unchanged. It is then available for multiple uses as a source of low cost cold.

The "COLD AG" concept was extensively explored by Dr. Martin Vitousek at the Keahole NELHA facility where deep ocean water is continuously pumped ashore from a depth of 2000 feet. Dr. Vitousek employed strawberries as his test crop and rediscovered the fact that the sugar content of the strawberry is a function of the difference in temperature between the fruit and the ground. Qualitative analysis of the crop established that yields from the cold beds were indeed sweeter than the experimental controls. These observations were in accord with experimental results by R. Gannmore-Neumann and U. Kafkafi (1983).[1]

Encouraged by these early results, he experimented successfully with a number of other spring crops such as gourmet lettuces, asparagus, and a Swiss ornamental flower, *Alstroemeria*. Following the work of Vitousek, Common Heritage Corp. collected anecdotal information on the role of cold in sugar and protein production. For example, it was observed that, in temperate climates, most fall fruits have a surface which is ideal in color and texture for heat rejection to a cold atmosphere and that the colder the atmosphere, up to the point of freezing, the sweeter the fruit. It was also noted that cacti which are not exposed to night cold do not flourish, and, of particular significance, that high quality straw mushrooms require a significant period of exposure to extreme cold (4° to 6° C). Additionally, many hydroponic systems are found to require cold nutrient fluids, and, finally, lettuce is known to thrive in 100° F weather as long as the soil is kept cool.

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[1] Ganmore-Neumann, R. and U. Kafkafi. 1983. The effect of root temperature and NO_3^-/NH_4^+ ratio on strawberry plants I. Growth, flowering, and root development. *Agron. J.*, **75**:941-7.

Common Heritage Corp. has employed crude thermodynamic-biochemical models focusing on the significance of ΔT induced by the selective application of cold to attempt to explain these observations. However, it is evident that a more sophisticated examination of the role of thermodynamics in plant physiology is needed. These speculations led to discussions with Dr. Ellen Weaver, a plant physiologist noted for her work in photosynthesis. At the outset of this workshop she described her preparation in terms of a review of the literature on the effect of root temperature on fruit production. She found a great deal of literature on cold tolerance, cold adaptation, cold resistance and cold hardiness but the plant physiologists were really not a great deal of help on the subject of the workshop suggesting that we are plowing new territory. She pointed out that at the site of photosynthesis, temperature does not play a role. In well-known studies Blackman has pointed out that photosynthesis at low light levels is independent of temperature, but it increases with high intensity. This is compatible with the notion that chemical reactions are speeded up at high temperatures. Indeed the conclusion is very strong that high temperature at the photosynthetic site is desirable. A workshop conclusion that cold root temperature is also desirable might lead to the speculation that high ΔT is the direction of optimum production with the further suggestion that thermodynamic processes might somehow play a role in the transport of phosphates and nitrates to the photosynthetic sites. It is for these paradoxes and reasons that the workshop participants were encouraged to bring their own understanding to a colloquium which openly and freely discussed both theoretical and practical aspects of this new field of agricultural endeavor, "COLD AG."

Low cost cold is, of course, also of great value in food processing and storage. A number of commercial enterprises at the NELHA facility are already finding extensive application of the cold in these areas. Considering the economics of low cost cold at Ke-ahole Point, therefore, it appears that the potential of "COLD AG" as well as other related applications of the cold is well worth pursuing and could play a significant role in Hawaii's attempts to pursue economic diversification.

In retrospect the workshop had four distinct phases:

a) the evaluation of the state of knowledge and significance of the role of cold and ΔT in plant physiology

b) an evaluation of the significance of low cost cold in commercial production processing and handling based on workshop site visits to NELHA,

c) an evaluation of the state of knowledge and significance of the role of cold and ΔT in fungi physiology and

d) an overall evaluation of the environmentally sustainable development significance of the use of deep ocean water as a major world resource.

The opening keynote speech was therefore that of Dr. Cary Mitchell who presented his paper "Fundamentals of Chilling Cold and Heat Stress in Plant Physiology."

John P. Craven
"COLD AG" Workshop Co-Chair

FUNDAMENTALS OF CHILLING COLD AND HEAT STRESS IN PLANT PHYSIOLOGY

By Dr. Cary A. Mitchell
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When I first was asked to make this presentation, I thought I merely would be reviewing biochemical and biophysical effects known to take place in plant tissues at the temperature of deep seawater, which is in the middle of the so-called "chilling" range of temperatures. Chilling temperatures are those above the freezing temperature of water and up to 10°C (50°F) to 12°C (53.6°F), and occasionally to 15°C (59°F). Plant species that have evolved to grow in tropical or sub-tropical climates would not tolerate exposure to chilling temperatures for very long, either in the root or shoot zones. This is why Chicquita *[sic]* Banana, in her TV commercials, sang "never put bananas in the refrigerator." They accumulate acetaldehyde and ethanol and rapidly turn brown and mushy, even without freezing. So you would not use cold seawater to grow tropical species such as banana or pineapple, but you don't have to. They are right here, and they grow quite well in warm, tropical soils. In fact, they require them. What you are interested in at this conference are temperate zone crops for which there is strong consumer demand but which are expensive to fly in from the mainland.

One reason temperate zone crops wouldn't do well here, under normal conditions, is that they would perceive tropical air and soil temperatures as "heat stress." Temperate species would not do well here for the opposite reason that tropical species wouldn't do well in a continental, temperate climate. Tropicals on the mainland would perceive it as "cold stress," at least during certain times of the year. As a matter of fact, most temperate species should even perceive the temperature of deep seawater as being growth limiting, and would not grow as well as they would if temperatures were above the chilling zone but below the heat-stress zone. Temperate species would not, however, suffer the irreversible damage that occurs with chill-sensitive species exposed to prolonged cold, but they would not perform productively, either. Minimum, optimum, and maximum temperatures exist for every species, and vary widely between species (Figure 1). This particular graph indicates effects of root temperature on shoot growth at a constant shoot temperature.

From a plant physiological point of view, the thing that is neat about this graph is that only roots have to be cooled to adapt at least some temperate species to a tropical climate. The crop I am most familiar with in this regard is leaf lettuce, which can be grown hydroponically, with roots maintained at a different temperature than that of the shoots. We stumbled on this phenomenon accidentally years ago while constructing our first recirculating hydroponics system. We were holding shoots of lettuce plants constant at 25°C in a controlled environment, but we made the mistake of putting a submersible pump in each nutrient solution reservoir. The pump motor heated up the nutrient solution that it subsequently pumped to troughs where plants were growing. The root temperature finally equilibrated at about 34°C (93.2°F), which was far

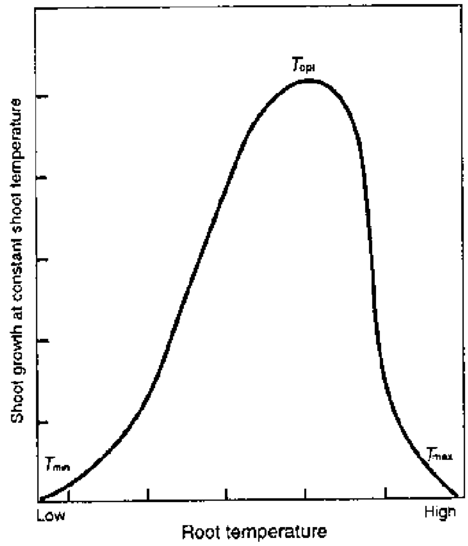


Figure 1 A generalized temperature response profile indicating minimum, optimum, and maximum root temperatures for growth of shoots.

too hot for lettuce, because it stunted shoot growth drastically compared to unheated solutions. We subsequently put cooling coils in each reservoir to counter the heating effects of the submersible pumps, and found that rootzone temperatures above 29°C (84.2°F) limited lettuce growth, with the higher the temperature the worse the effect. We don't use submersible pumps for recirculating hydroponics systems anymore, and we find that nutrient solutions equilibrate at about the same temperature as air.

In Arizona, they frequently are faced with hot air temperatures, often exceeding 100°F (37.8°C) during the summer. When they tried to grow lettuce hydroponically, it bolted and developed the bitter principle at those temperatures. However, if nutrient solutions were cooled to 65°F (18.3°C), it didn't matter if the shoots were at a daytime air temperature of 95°F (35°C), or even hotter. The root-cooled plants didn't bolt and they didn't become bitter, either. They developed normally. Note that as root zone temperatures increased from 65°F to 95°F, root growth was relatively unaffected up to 85°F (29.4°C), but at 95°F it was greatly inhibited. For whatever reason, this root-cooling technique did not catch on, and hydroponic production of lettuce with root cooling did not become commercialized in Arizona.

These observations with lettuce plus your observations with strawberry suggest that rootzone temperatures are of primary importance in determining whole plant response to temperature. However, the sample size is still small, and we need to be careful lest we be tempted to make premature, sweeping generalizations about all plant species before adequate research has been done. Nevertheless, we have every reason to believe that roots are very important in determining responses of whole plants to extreme temperatures, be they high or low. So, what are the likely candidates to explain known temperature responses?

The most obvious plant processes to come to mind are growth, photosynthesis, and aerobic respiration (Figure 2). For all of these processes there is a minimum or threshold temperature, an optimum temperature range, and an upper limit or maximum temperature, above

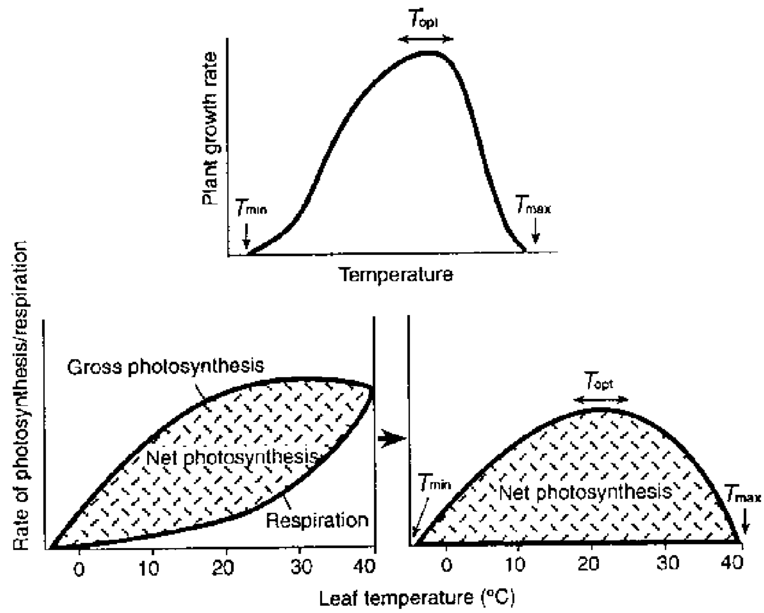


Figure 2. Generalized plant reponses of growth, photosynthesis, and respiration to a range of temperatures.

which is the thermal death point. These three are the so-called "Cardinal Temperatures" of plant processes. Photosynthesis and shoot respiration have different Cardinal temperatures, and although this particular graph doesn't show T_{max} for respiration, it is well above 40°C in this example. T_{opt} for gross photosynthesis is between 20 and 25°C, above which it plateaus or goes down slightly. Since photosynthesis and respiration are opposite processes with respect to gas exchange, net photosynthesis ends up with a much lower T_{max} than does respiration *per se* (Fig. 3). In this extended temperature profile of photosynthesis and respiration, respiration predominates at temperatures above the heat limit, and the plants actually would lose biomass. Notice that both processes are slow at the temperature of deep seawater.

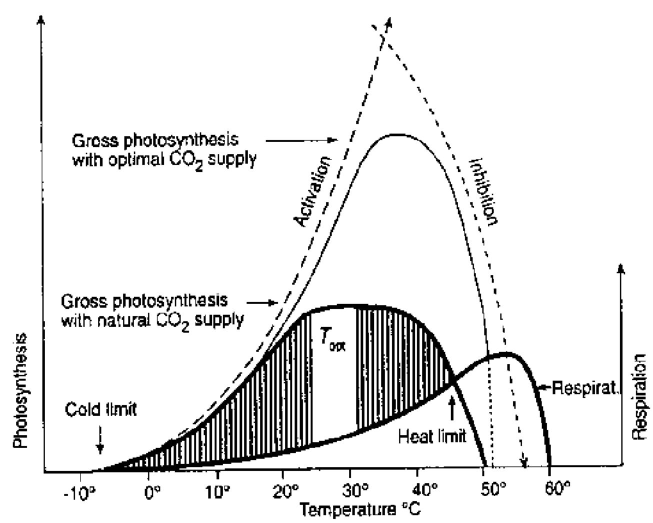


Figure 3. Temperature response of photosynthesis and respiration.

Table 1. Cardinal Temperatures (°C) for Net Photosynthesis At Light Saturation

Species	T_{min}	T_{opt}	T_{max}
Tropical C4 plants	5-7	35-45	50-60
C3 crop plants	-2 to 0*	20-30	40-50
Sun plants	-2 to 0	20-30	40-50
Shade plants	-2 to 0	10-20	ca. 40
Alpine plants	-7 to -2	10-20	30-40
Tropical evergreens	0-5	25-30	45-50
Temperate deciduous	-3 to -1	15-25	40-45

Tropical day: 35°C, 90% RH — Temperate day: 22°C, 61% RH
Tropical night: 30°C, 94% RH — Temperate night: 14°C, 95% RH

Let's compare Cardinal Temperatures at light saturation levels for a number of different plant species (Table 1). These temperatures are highly correlated with the climates these species are indigenous to. High efficiency C4 plants barely function at the temperature of deep seawater; peak performance is at 35-45°C (i.e., 95 to 113°F); and T_{max} is 50-60°C (i.e., 122 to 140°F). Less efficient C3 species function all the way down to the freezing point of water, but not much; they generally peak at 20 to 30°C (i.e., 68 to 86°F), and they die above 40 to 50°C (i.e., 104 to 120°F). Sun and shade plants are similar to C3 plants; in fact, many of them are C3. The preferences for shade plants are a little cooler than for sun plants. Everything is shifted down, of course, for alpine species. Cardinal temperatures for tropical woody species tend to be a little lower than those for tropical herbaceous species. Temperate woodys are shifted down still further from the tropicals, as you would expect. Incidentally, a typical tropical day might be 35°C (i.e., 95°F) and very humid. Tropical nights don't cool off that much (i.e., 86°F) and are even more humid. I suspect Hawaii's climate is more pleasant than this reference tropical day and night. Temperate climates vary all over the place, but a reference climate during the growing season might have a moderate temperature and be less humid during the day. It can cool off considerably at night, but this causes nighttime relative humidity to soar.

In non-photosynthetic tissues such as roots, respiration provides the reducing power and energy needed for maintenance and growth of those tissues (Figure 4). As growth rate increases, total respiratory rate also goes up. However, as ambient temperature goes up, the amount of total respiration needed to maintain the health and integrity of tissues also increases even before any growth can be accommodated. This "maintenance" respiration becomes the major portion of total respiration beyond T_{opt}, and so-called "growth" respiration contributes proportionally less. Above T_{max} for maintenance respiration, membranes deteriorate, cellular compartmentalization disintegrates, even respiration stops, and the plant dies. At chilling temperatures, the ratio of growth to maintenance respiration actually is quite efficient, providing the species in question is not damaged by chilling. The total amount of respiration and growth still may be quite low at chilling temperatures, however.

Temperature coefficients also can be useful indicators of the efficiency of certain plant responses to changing temperature in the physiological range (Table 2). The ratio of the rate of a

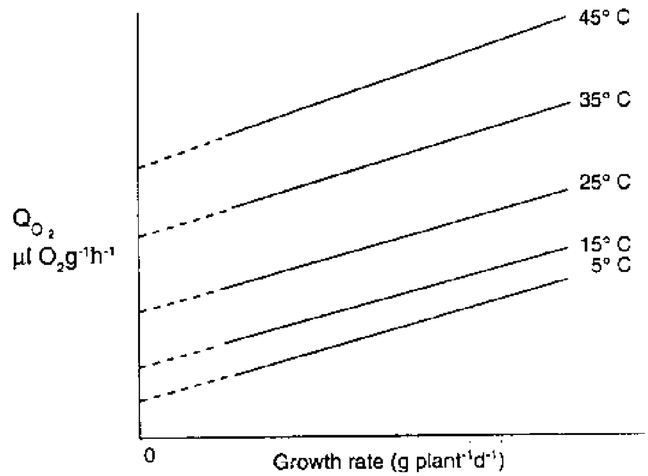


Figure 4. Maintenance respiration (y intercept) and growth respiration vs. growth rate at different temperatures.

process at a given temperature over the rate of the same process at a 10°C lower temperature is called Q_{10}. Q_{10} values for diffusion of small molecules in liquid indicate that this physical process is only slightly temperature sensitive, whereas enzymatic and active transport processes are highly temperature responsive. The light reaction of photosynthesis, of course, is a photochemical process, so it is temperature insensitive in the physiological range, whereas the dark reaction is enzymatic and its Q_{10} indicates that it is very temperature responsive. Because the light reaction is so fast in this temperature range, it is not limiting to photosynthesis when light is saturating. Thus, the overall process of photosynthesis is temperature dependent because of the dark reaction. A good example whereby the Q_{10} of a physical process can become limiting to a process is the internal breakdown disease of potato tubers stored at too high a temperature. Because of their low porosity, oxygen diffuses to the interior of tubers much slower than it is used up by the respiration of overheated tissues. Thus, O_2 becomes depleted within the tuber, anaerobic respiration predominates, and fermentation products build up in the tubers and damage them.

Table 2. Temperature coefficients (Q_{10}) of plant processes within the range 0 - 30°C.

Process	Q_{10}
Diffusion of small molecules in H_2O	1.2 - 1.5
Enzymatic hydrolysis reactions	1.5 - 2.3
Respiration	2.1 - 2.6
Photosynthesis (light reaction)	~1
Photosynthesis (dark reaction)	2 - 3
Potassium uptake into roots	2 - 5

$$Q_{10} = \frac{\text{rate at temperature T + 10°C}}{\text{rate at temperature T}}$$

There is yet another adaptation of mitochondrial respiration to changing temperature that is noteworthy (Figure 5). The usual terminal electron acceptor of mitochondrial respiration is cytochrome oxidase, whose action can be inhibited by cyanide. If respiring tissues are exposed to prolonged cold, an alternative, cyanide-insensitive respiration may be induced whose function in plants is not well understood. Alternative respiration can be inhibited by substituted hydroxamic acids, such as SHAM. Alternative respiration also is less coupled to ATP formation than is the cytochrome pathway; it has less affinity for oxygen; and sometimes it is thermogenic, meaning that it gives off heat. Alternative oxidation is an adaptation to cold temperature, but thus far it is not known whether it plays a role in the chill resistance of plants to cold.

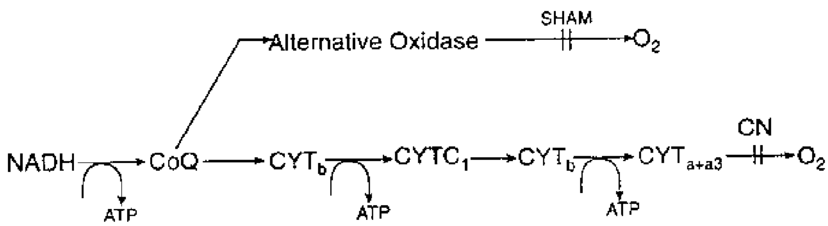


Figure 5. Cytochrome and alternative oxidases of mitochondria using oxygen as a terminal electron acceptor.

On the other end of the temperature stress spectrum, hot temperatures induce "heat shock proteins" (HSPs), in plant tissues that are not adapted to heat (Table 3). HSPs accumulate in heat-stressed tissues in response to a number of treatments. An optimum condition for HSP induction in higher plants is a drastic upshift from a moderate temperature to 39-41°C. HSPs also can be induced by a gradual temperature increase of 2.5°C per hour. HSP synthesis usually can be detected within 20 minutes of initiating heat shock treatment. An increase in the transcript levels of some HSP genes has been shown to occur in as little as 3-5 minutes after initiating heat stress. Even if the heat shock treatment is maintained continuously, HSP induction is transient. Just as for alternative respiration, a role for HSPs has not been established. It has been speculated, however, that HSPs may play a role in establishing thermotolerance of cells. That doesn't mean HSPs make intolerant species more thermo-tolerant, however. Plants generally try to adapt to environmental stresses; some are just better at it than others. I would not expect native plants in Hawaii to develop HSPs, unless the weather became unseasonably hot. Plants introduced to the tropics from temperate climates, however, might undergo the heat shock response if it gets

Table 3. Heat shock response in plants.

Heat Shock Response in Plants
• Heat shock proteins (HSPs) induced by... ... drastic upshift to 39 - 41°C ... increase of 2.5°C per hour
• HSP synthesis detectable in 20 minutes
• Transcript levels increase in 3 - 5 minutes
• HSP induction is transient
• Role of HSP in establishing thermotolerance of adaptable plants?

hot enough, but this wouldn't be expected to adapt them to a tropical climate. It would be interesting to determine whether root zone cooling of temperate crops would negate the heat shock response in shoots. I am not aware that this yet has been investigated.

One of the more graphic examples of differences in plant response to temperature extremes is the difference in tissue metabolism exhibited by chill-resistant and chill-sensitive species (Figure 6). If the natural logs of reaction rates are plotted against the inverse of temperature, the slope of the resulting straight line indicates the activation energy of the metabolic process being graphed. Respiration rates of tissues or mitochondria are favorites for this type of plot. This particular example is for exudation rates from detopped plants. Reaction rate typically declines with decreasing temperature, but the activation energy for the reaction remains constant over the entire range of chilling and non-chilling temperatures for a chill-resistant fruit such as apple. However, for a chill-susceptible fruit such as banana, these Arrhenius plots, as they are called, would indicate a distinct break in slope of the straight line at some "critical" temperature in the chilling range, below which activation energy for the reaction is greater than at temperatures above the critical temperature. The temperature at which the discontinuity occurs in an Arrhenius plot indicates that an impairment occurs in cellular membranes with which the reaction being measured is associated. The temperature of the discontinuity is thought to reflect a "phase change" from a typical liquid-crystalline state above the break point to an atypical solid gel state below the break point. The ratio of saturated-to-unsaturated fatty acids and the amount of sterol in the lipids of membranes largely determine the critical temperature for phase change. In general, the higher the content of unsaturated fatty acids, the lower the temperature at which phase change will occur, if at all. Chill-resistant fruits like apple do not have a phase transition in the chilling range, but banana does. In banana, phase change means that membrane-associated respiratory reactions in mitochondria become disabled relative to non-membrane-bound, soluble

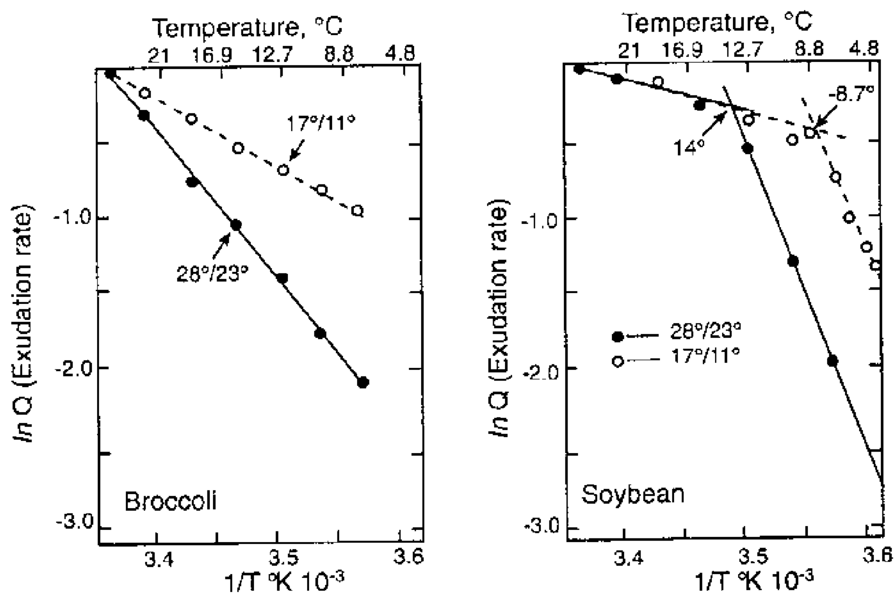


Figure 6. Effects of temperature on rate of water movement through broccoli and soybean root systems grown under different day/night temperature regimes: 28°C day/23°C night vs. 17°C day/ 11°C nights.

reactions, whose enzymes have a lower activation energy than do the impaired membrane ones. Unfortunately for tropical fruits, the soluble respiratory enzymes catalyze anaerobic, fermentative reactions, whose products cause tissue browning and deterioration. Chilling the tissues of tropical plants would cause responses analogous to those in chill-sensitive fruits, and this determines their cold intolerance. Roots of strawberries should be like apples: their lipids should be more unsaturated and there should be no phase change in the chilling range. Cold will greatly inhibit respiration and metabolism of strawberry roots, most likely to the point of being growth-limiting, but it shouldn't injure strawberry roots because they should be chill-resistant.

The two species in the graphs illustrate well the differences between chill-resistant and susceptible species. Broccoli represents a chill-resistant species. Regardless of whether broccoli is grown under a warm or a cool day/night temperature regime, there is no break in the Arrhenius plot between 21° and 5°C. The rate of water movement through stems of cool-grown broccoli plants declined more slowly with decreasing temperature than it did for warm-grown plants. The activation energy for warm-grown broccoli was a little more than for cool-grown. This indicates some adaptation to cool temperatures. Soybean, on the other hand, is a chill-sensitive crop. When grown under 28°C days/23°C nights, a phase transition occurred for exudation rate at a relatively high chilling temperature of 14°C. However, if grown at 17°C days/11°C nights, the plants harden enough to resist phase change all the way down to 8.7°C, below which activation energy for the reaction is greatly enhanced.

The key event in chilling injury of intolerant species is the membrane phase transition (Figure 7). Not only are activation energies for membrane-associated reactions increased below the discontinuity, but protoplasmic streaming ceases and membranes become leaky to ions and other solutes. If chilling doesn't go on too long, effects can be reversed and plants will recover. But if exposure is prolonged, metabolism will be upset, ATP supply will decline, and fermenta-

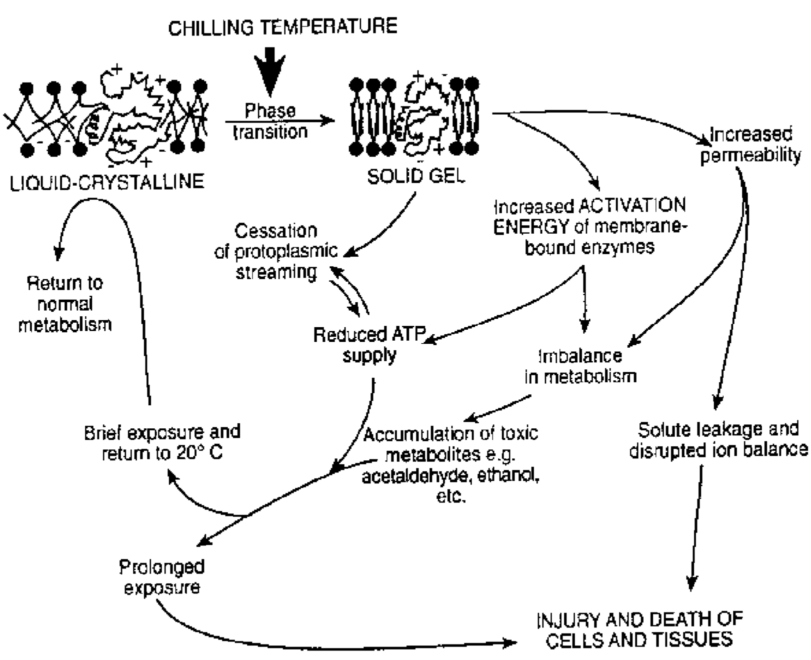
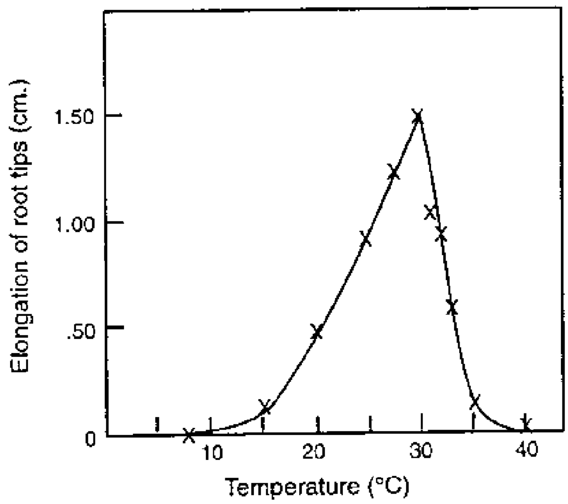


Figure 7. Events leading to chilling injury in sensitive plant tissues.



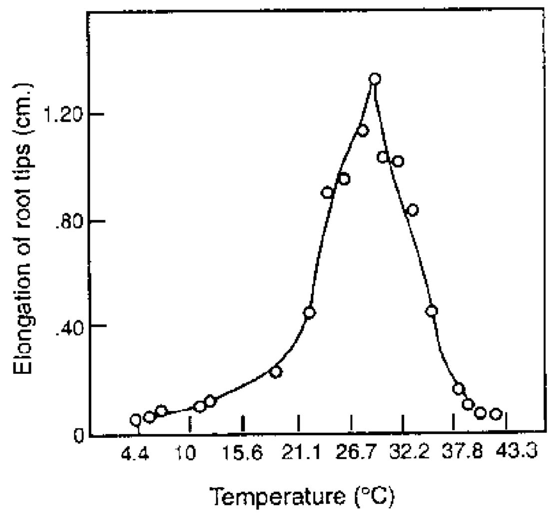


Figure 8. Growth of excised root tips of tomato (left) and pineapple (right) over a range of temperatures from chilling to heat stress.

tion products will accumulate. All of these things contribute to the eventual injury and death of cells and tissues. Once again, species whose membranes have enough unsaturated fatty acids to avoid phase change in the chilling range of temperatures will not suffer the symptoms and effects of this disorder.

With our reinforced appreciation for chilling and heat shock limitations fresh in mind, let's examine a few examples of root growth profiles over a wide temperature range for tropical and temperate species (Figure 8). Tomato and pineapple are warm-weather species with T_{opt} for root growth a very definite 30°C or higher. Neither species likes temperatures above 40°C, or anywhere in the chilling range. Optimum and maximum temperatures for root growth of a temperate zone pine are 5°C lower than those for tomato and pineapple, but growth at 15°C is substantially more than for the other two (Figure 9). However, at 5°C, the story is the same for all three species. Once again, even the roots of chill-resistant species don't grow significantly in the mid-chilling range, even if they aren't injured.

Thus far, we have seen how different temperatures can affect vital processes such as respiration by effects on membranes, on activation energies, on rates of reactions as manifested by Q_{10} values, and on different types of respiration that take place. Another subtle, but not insignificant, one is the effect of temperature on availability of oxygen for aerobic respiration (Table 4). One manifestation of this availability could be the solubility of O_2 in water at different temperatures. If soils are very warm, for instance, and roots are

Table 4. Solubility of oxygen in water saturated with air at 1 atmosphere of pressure.

Water Temperature (°C)	(°F)	O_2 Solubility (ppm)
0	32	14.6
5	41	12.8
10	50	11.3
15	59	10.2
20	68	9.2
25	77	8.4
30	86	7.7
35	95	7.1
40	104	6.5
45	113	6.0
50	122	5.6

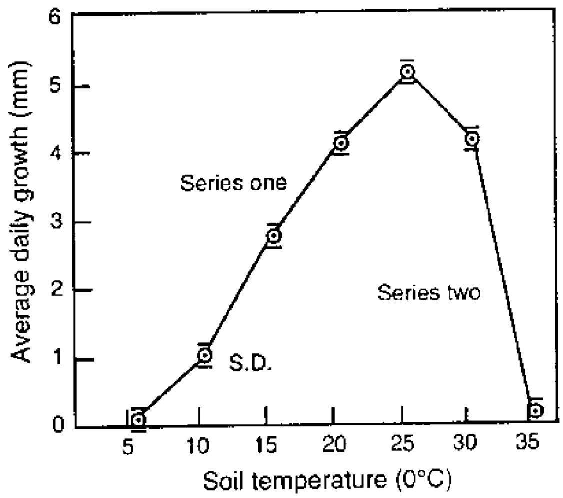


Figure 9. Relationship between soil temperature and rate of root growth of Pinus taeda *seedlings.*

trying to respire rapidly because it is warm, the declining solubility of O_2 in soil water wouldn't help. Although O_2 is at 21% or 210,000 ppm in air, it ranges only from 15 down to 5 ppm in water between 0 and 50°C, the O_2 solubility changing inversely with changes in temperature. This factor becomes important in wet soils, where the pore spaces are mostly filled with water, or where they are not continuous to the surface to allow for good gas exchange. In fact, I have been wondering how wet these "selfwatering" soils become using the "COLD-AG" technique of constantly chilling the soil surface below the dewpoint with balmy tropical air above. Logic would suggest that the growing medium should be well-drained and highly porous with low bulk density so that root hairs would maximize contact with air spaces and minimize the chance of waterlogging. The potential importance of this oxygen solubility table would be greatly diminished if those precautions are taken. This table is very important for hydroponic culture of plants, however. If all of the O_2 for root respiration has to come from 8 ppm or less O_2 in a nutrient solution, procedures for continuous aeration become very important. Half again more O_2 is available in solution at 5°C than it is at 25°C. For crops that are not subject to chilling injury, the extra O_2 in cool soils can't hurt anything.

Yet another important effect of temperature on plants pertains to water relations (Figure 10). A direct effect of temperature is on the viscosity of water, both in the soil as well as in the plant. The reciprocal of viscosity is the fluidity of a liquid. The viscosity of liquids increases with decreasing temperature. For example, the viscosity of water doubles as temperature drops from 25°C to 0°C. As indicated in this figure, the rate of water flux through roots changes in a linear manner across the physiological range of temperatures, with progressively less getting through as soil or water gets cooler. In living roots, the viscosity situation is complicated by the effect of temperature on permeability of cells to water uptake and hydraulic conductivity through the entire root system. By using dead roots, the permeability issue is eliminated and water flux through roots varies with temperature in almost the exact way that water viscosity changes with temperature. The fact that water flux through living roots is less than that through dead roots at low temperatures and more at higher temperatures reinforces the importance of temperature on membrane fluidity and metabolism regulating water permeability. This figure (Figure 11) illustrates the relative increases in water viscosity and resistance to water flow through plants as

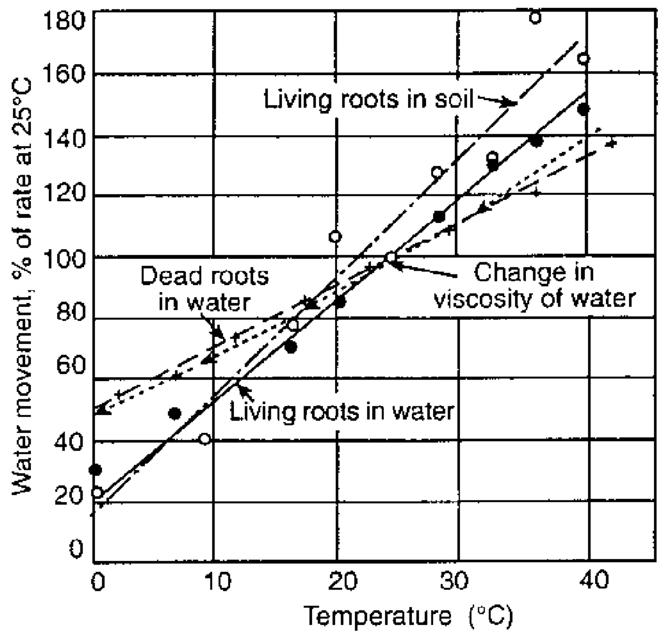


Figure 10. Combined effects of decreased permeability of root cells to water and the increased viscosity of water with decreasing temperature on water flow through roots.

temperatures fall through the chilling range. The comparison is made for a temperate zone spruce and a sub-tropical citrus. For the chill-resistant spruce, resistance to flow doubles with 10°C of cooling from 12°C to 2°C. About half of that resistance change can be attributed to increased viscosity of water *per se*. A 10°C decline in soil temperature of a chill-sensitive species causes a more than 3-fold increase in resistance with a change in water viscosity similar to the spruce. Clearly, low temperature causes a much more significant lesion with respect to water flux for chill-sensitive species than for chill-tolerant species.

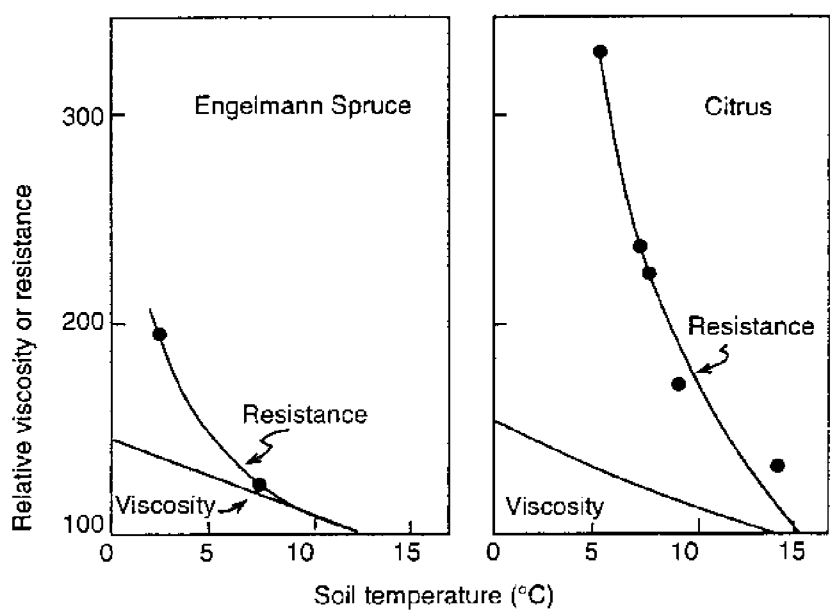


Figure 11. Relative viscosity of water and relative resistance for water flow from soil to leaves as a function of soil temperature.

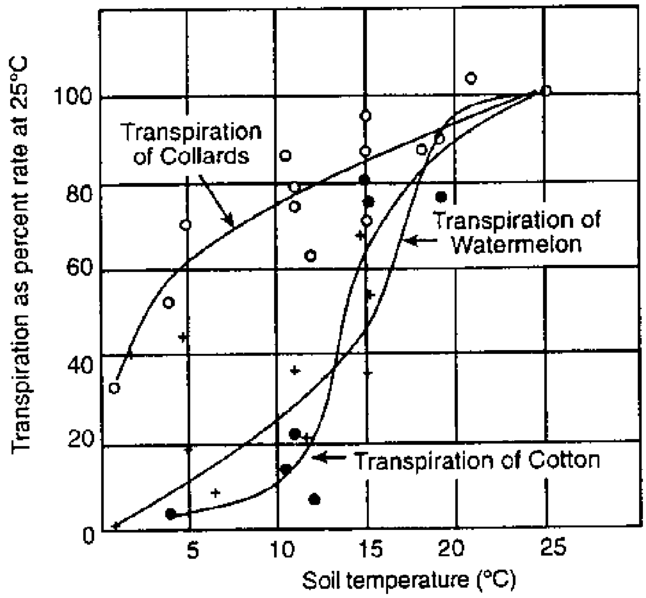


Figure 12. Effects of soil temperature on transpiration by collards (cold season) and cotton and watermelons (warm season).

I would like to suggest that indicator species such as sunflower, which, when detopped, can sustain delivery of large volumes of xylem exudate by root pressure for many days from the cut stump, be used for "COLD AG" research and development to determine cooling temperatures that will minimize lesions to root water relations.

Let's extend the influence of soil temperature, now, to transpiration of water vapor through stomatal pores in leaves (Figure 12). Transpiration is a whole-plant function involving a combination of effects of driving forces on water evaporation from leaves as well as root pressure and water permeability. Here, two warm-season crops, cotton and watermelon, are contrasted with collards, a cold-season crop, with respect to relative transpiration rates. First of all, note that decreasing soil temperatures inhibit transpiration of all three species. Even the transpiration of collards is inhibited 40% by lowering soil temperature from 25°C to 5°C. The effect is gradual and linear all the way down to 5°C, below which it nosedives, however. In contrast, the warm-weather crops start declining precipitously at temperatures below 20°C. Watermelon is even more sensitive than cotton, but cotton catches up as temperatures fall into the chilling zone. Both warm season species are 90-95% shut down at 5°C, compared to 40% for collards. Plants growing in too cold a soil likely will wilt, especially if the shoot environment is sunny and warm. The decreased transpiration rates shown in the figure could be due either to decreased ability of the cold root system to deliver water to the shoot, or to stomatal aperture reduction, or to both.

The next temperature-responsive factor I would like to discuss is modified phytohormone biosynthesis and distribution within plants (Figure 13). This likely is a major factor in how temperature-stressed roots signal shoots regarding their physiological condition. One of the best known responses to heat stressing roots is an inhibition of cytokinin biosynthesis by root tips. Cytokinins synthesized in roots typically are transported to leaves and meristems via the xylem transpiration stream. Disruption of this cytokinin supply by heat stress could result in chlorosis,

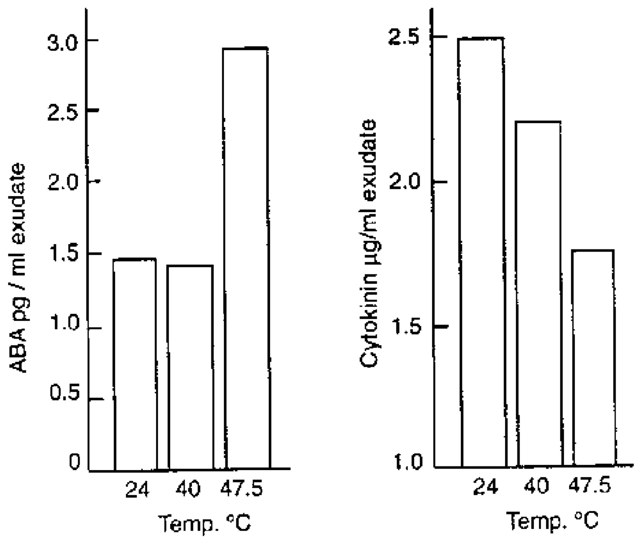


Figure 13. Effect of brief heat shock to roots on ABA and cytokinin contents of xylem exudate of bean plants.

premature senescence, impaired cell division, stomatal closure, and stem bolting. It would be useful to learn whether some of the adverse effects of tropical climates on growth of temperate crops could be ameliorated by applying exogenous cytokinins to the shoots of heat-stressed plants. This includes trying to prevent the bolting of lettuce under heat stress conditions without cooling roots. Root tips also synthesize abscisic acid and a 2-minute heat shock treatment applied to beans doubled the usual level of ABA in xylem exudate going to the shoot (Table 5). Interestingly, chilling also promotes ABA biosynthesis by a number of warm-season crops, including bean, so either temperature extreme can turn on ABA synthesis in this species. Under either circumstance, ABA tries to re-establish water balance of stressed plants, both in roots and shoots.

Table 5. Summary of temperature-stress effects on phytohormone biosynthesis and distribution.

TEMPERATURE-STRESS EFFECTS ON PHYTOHORMONE BIOSYNTHESIS AND DISTRIBUTION
• Heat shock-induced inhibition of cytokinin biosynthesis by bean and tobacco roots
• Heat shock-induced promotion of abscisic acid biosynthesis by bean roots and peas
• Chill-induced promotion of free and conjugated forms of abscisic acid by warm-season crops and wheat
• Chill-induced promotion of gibberellin biosynthesis by long-day, biennial, vernalizable, rosette species
• Chill-induced promotion of ethylene precursors in cucumber fruits

In roots, ABA enhances hydraulic conductivity and uptake of cations such as potassium. In shoots, ABA induces stomatal aperture reduction and reduces leaf wilting. Obviously, not all species are successful with these attempts to adapt. Application of the ABA biosynthesis inhibitors fluridone or norflurazon would provide additional useful information regarding the role of this hormone in cold responses and adaptation.

Gibberellins are well-known to induce bolting in long-day or cold-vernalizing species. It also is known that chilling, which itself induces bolting, greatly enhances the content of endogenous GAs in those plants just before bolting. Root tips are known to also synthesize gibberellins, but the impact of root chilling on non-bolting species is uncharacterized. Heat-induced promotion of GA biosynthesis also is obscure; I'm not sure if anyone has tried to prevent the heat-induced bolting of lettuce with the GA biosynthesis inhibitors CCC or B-9, but it would be so easy to do, that someone ought to try it.

Ethylene, of course, is the universal stress hormone of plants, and its evolution at an enhanced rate would be expected from chilled or heat-stressed tissues. In fact, exposing cucumber fruits to a temperature of 2.5°C accelerated the synthesis of ACC, the immediate precursor of ethylene. Roots are well known to synthesize ACC, and to transport it to shoots where it is converted to ethylene. Chilling or heating the roots of intolerant species could indirectly induce premature senescence, epinasty, and leaf abscission in the shoot mediated by stress ethylene production. As for the other hormones, there is a large arsenal of ethylene action and biosynthesis inhibitors that could be used to test the role of ethylene in plant responses to chilling or heat shock. I'm sure chill-sensitive, tropical species would generate stress ethylene in response to cold roots, but it also would be useful to learn whether chill-resistant, temperate species indicate some stress, when exposed to low temperature, especially that of cold seawater.

I would like to make a few closing comments about strawberry in this "COLD AG" setting that may have bearing on other species as well. Because strawberry is a small-statured plant whose crown and leaf canopy are no more than a few inches above the soil surface, shoot temperatures may not be all that much warmer than cold roots, even on a warm, sunny Hawaiian day. Several factors may contribute to shoot cooling of strawberry by cold roots (Table 6). For one thing, the water moving from the roots up into the shoot is cold, cooling the shoot from within. Furthermore, evaporative cooling occurs at transpiring leaf surfaces. On still days, or in wind-protected areas, a boundary layer of cool, moist air might build up and blanket a

Table 6. Factors affecting cooling of strawberry shoots in chilled soil.

FACTORS AFFECTING COOLING OF STRAWBERRY SHOOTS IN CHILLED SOIL
• Conductive cooling of tissues from below and from within
• Evaporative cooling from evapotranspiration
• Convective coating from a cool air boundary layer in still conditions
• Longwave radiation from leaf to cold, dark, wet soil as well as to sky

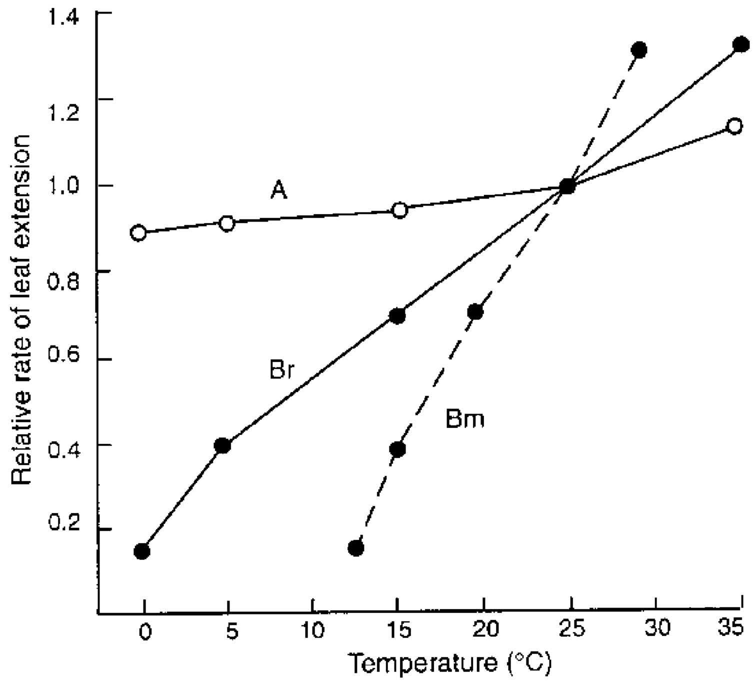


Figure 14. Effect of varying root temperature at constant air temperature ± controlling shoot meristem temperature of Zea mays.

root-chilled strawberry field like an inversion layer. Still another way shoot parts may become cooler than they would normally is by direct radiation of infrared or thermal energy from lower leaf surfaces to the cold, wet, darker-than-normal soil surface, in much the same way that leaves radiate heat to a clear sky at night. So for strawberry, at least, shoot tissues might not be as warm over a cold soil as would shoots of a larger-statured small fruit, such as raspberry. There even is evidence that a tall crop like corn has cool shoot meristem tissues at low soil temperatures, even if air temperatures are held constant at 25°C. An additional, thermostatically controlled collar placed just below the meristem assures the meristem remains at 25°C, and this wipes out the effect of soil temperature on leaf elongation (Figure 14). So cool internal water affects shoot tissue temperatures, even far above the soil line. Chilled shoots often have trouble closing stomates, which could further aggravate secondary water-stress symptoms that may arise, such as wilting.

Table 7. Factors affecting sweetness of cold ag strawberries.

FACTORS AFFECTING SWEETNESS OF COLD AG STRAWBERRIES:
• Reduced respiration by cool shoots • Sink demand of colder roots less than that of developing fruits • Cold-hydrolysis of starch to sugar

If the previous factors interact to keep the developing fruits of "COLD AG" strawberries cool, they would not burn up as much sugar in respiration as if they were hot (Table 7). Also, if roots are kept cooler

Table 8. A summary of optimum root temperatures for growth of strawberry plant parts.

PLANT PART	OPTIMUM TEMPERATURE	
	°F	°C
Roots	55	12.8
Crown	65	18.3
Leaf	75	23.8
Fruit	45	7.2

than shoots, root respiratory processes would be suppressed; the sink demand of roots for photosynthate, therefore, would be lessened, and more sugar would be available to accumulate in the fruits. Furthermore, in specialized storage organs such as potato tubers, cold storage is known to activate starch-hydrolyzing enzymes, resulting in accumulation of osmotically active sugars. If strawberries normally store some starch in the fruits, cold hydrolysis could be yet another factor contributing to the incredibly sweet fruits we are hearing about.

I ran across some fascinating information indicating that development of different plant parts of strawberries have different optimum rootzone temperatures (Table 8). Averaged over a number of different cultivars, root growth *per se* was found to be best at 55°F; crown growth was best at a moderate root temperature of 65°F; leaf growth was best at moderately warm root temperatures of 75°F, and fruit development was best at a quite cool root temperature of 45°F. The conclusion of this experiment is that there is no single optimum rootzone temperature for strawberry. Optimum crop performance would appear to require different root temperatures at different times of crop development and to be exceedingly complex. Special programming of rootzone temperatures over time should be possible with the "COLD AG" technique but would require some research to work out the right soil temperature to go with the Hawaiian climate. Temperature-controlled hydroponic culture could be a useful research tool if optimization of production for commercial purposes were a goal.

I would like to recommend, at the very least, that profiles of soil and tissue temperature be monitored, both with and without "COLD AG" treatments, at all times of day and night (Table 9). Air temperature, humidity, solar irradiance, and photoperiod also should be monitored to help analyze what's going on. Plant water relations and tissue temperatures need to be measured under a variety of conditions for each test species or cultivar. Transpiration rates and stomatal conductance should be measured using a leaf porometer; root exudate from cut stumps should be collected and measured for guttation rate, and an infrared thermometer should be used to measure tissue and soil surface temperatures under a variety of conditions. Different cultivars and species of strawberries should be compared and additional small fruits should be tested to help determine universality of the "COLD AG" effect. As for all experiments, uncooled control plots should be run concomitant with experimental treatments. Adequate replication and verification of results should be part of the experimental design.

Table 9. Summary of environmental and plant variables that must be measured in "COLD AG" research.

ENVIRONMENTAL AND PLANT VARIABLES TO BE MEASURED IN COLD-AG RESEARCH
• Temperature: soil, tissue, and air (day/night) • Humidity: relative, dew point temperature • Solar irradiance: PAR, total radiation • Photoperiod • Plant water status: transpiration rates, stomatal conductance, xylem exudation • Genotype differences in yield performance • Depth, spacing, and density of cold seawater pipes • Flux of seawater through pipes

If what I have suggested in this presentation about plants not performing optimally at the exact temperature of cold seawater is true, it should not be difficult to adapt "COLD AG" methodology to achieve "Cool Ag" conditions that still give the desired results, but with greater productivity. The depth and density of pipes below culture beds, as well as the flux of seawater through pipes, should be easily adjustable to create any rootzone temperature below ambient that is desired. I suspect the preliminary success reported with strawberry is due to a gradient of lessening cold with distance above the buried seawater pipes. The steepness of this temperature gradient would be affected by solar heating of the soil surface and the actual temperature of the rootzone is determined by the opposing effects of thermal conductance downward and cold conductance upward. Without solar warming to counter cold seawater, soils would become maximally cold at night. Continued chilling after sunset may be unnecessary, or even undesirable for some species or cultivars.

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MUSHROOM AND MUSHROOM CULTIVATION

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Introduction

During the past decade there has been a great increase in the popularity and production of mushrooms throughout the world and particularly in Southeast Asia. This has been due, in part, to a growing awareness that mushrooms, apart from their delicacy, have a high nutritional value, possess important tonic and medicinal properties, and can serve as a cheap source of protein. Another contributing factor has been the financial assistance and additional support from United Nations agencies for conducting training courses, workshops and conferences on mushroom research and production in many Asian countries.

What is a mushroom?

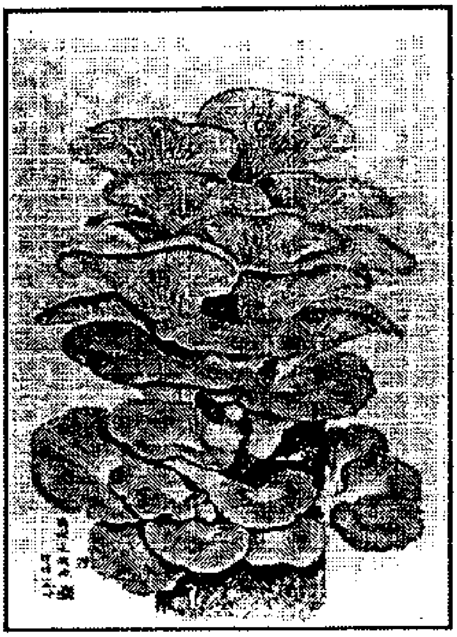
This is not a new question or a new issue. The word mushroom may mean different things to different people and different countries. Specialized studies, and the economic value, of mushrooms have reached the point where a clear definition of the term 'mushroom' is now warranted. This will be of considerable benefit at a time when the number of cultivated mushrooms species is rising, when production of established cultivated mushrooms continues to show a steady expansion (Figure 1), and when an increasing number of countries and people are engaged in mushroom cultivation as an agricultural or industrial technology. In this report, 'mushroom' is defined in the broad sense as "a macrofungus with a distinctive fruiting body which can be either epigeous or hypogeous and large enough to be seen with the naked eye and to be picked by hand" (Chang & Miles, 1992). Thus, mushrooms need not be Basidiomycetes, nor aerial, nor fleshy, nor edible. Mushrooms can be Ascomycetes, grown underground and have a non-fleshy texture. Mushrooms can be divided into four categories:

(1) those which are edible and fleshy and fall into the edible mushroom category, e.g. *Agaricus bisporus;*

(2) those which are considered to have medicinal applications and are referred to as medicinal mushrooms, e.g. *Ganoderma lucidum;*

(3) those which are proven to be, or suspected of being, poisonous e.g. *Amanita phalloides;*

(4) a miscellaneous category which includes a large number of mushrooms whose properties remain less well-defined and which may tentatively be grouped together as 'other mushrooms.' This form of classifying mushrooms is not absolute; many kinds of mushrooms are not only edible but also possess tonic and medicinal properties.



Pleurotus sajor-caju (Fr.) Sing.

Drawing by Mao Xao Lan, 1990.

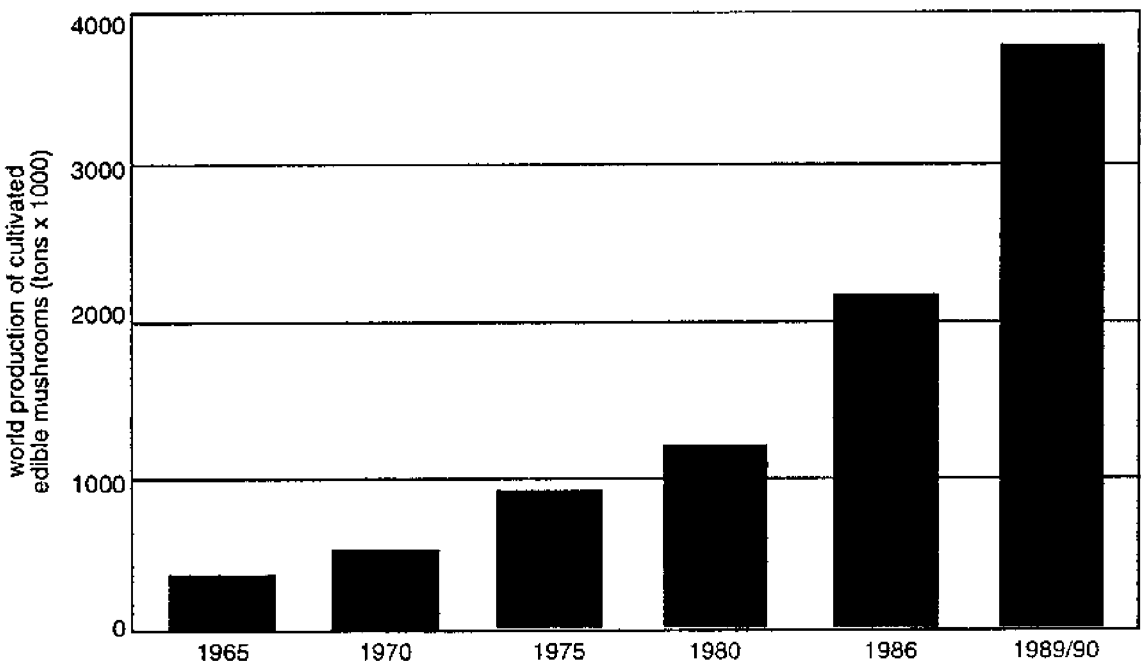


Figure 1. Annual world production of cultivated edible mushrooms.

Magnitude of mushrooms

Although the number of known species of fungi is put at between 69,000 to 80,000, it is conservatively estimated that 1.5 million species exist (Table 1) (Hawksworth, 1991). The fungi are regarded as the second largest group of organisms in the biosphere after the insects, estimates of which range between 10-80 million (Stork, 1988). Thus, known fungal species constitute only about 5% of the estimated total and, therefore, the large majority of fungi are still unknown. Out of 80,000 described species of fungi, there are about 10,000 species of fleshy macrofungi and only a handful of these are lethal. There are no simple ways of distinguishing between the edible and the poisonous and a mushroom should only be eaten if it can be identified with precision. About 2,000 species from more than 30 genera are regarded as prime edible mushrooms but only about 80 of these are grown experimentally, about 40 are cultivated economically, and only 4 to 5 are produced on an industrial scale (Chang, 1990).

Table 1. Comparison of the numbers of known and estimated total species in the world of selected groups of organisms.

Group	Known species	Total species	Percentage known (%)
Vascular Plants	220000	270000	81
Bryophytes	17000	25000	68
Algae	40000	60000	67
Fungi	69000	1500000	5
Bacteria	3000	30000	10
Viruses	5000	130000	4

Source: Hawksworth (1991)

History

Prehistoric man almost certainly used mushrooms as food and there is ample evidence that the great early civilizations of the Greeks, Egyptians, Romans, Chinese and Mexicans prized mushrooms as a delicacy, for purported therapeutic value and, in some cases, for use in religious rites. Throughout recorded history, there is repeated reference to the use of mushrooms as food and for medicinal purposes, and it is therefore not surprising that the intentional cultivation of mushrooms had a very early beginning. We now know that this occurred in China around 600 A.D. with the cultivation of *Auricularia auricula* on wood logs (Chang & Miles, 1987). Other wood-rotting mushrooms such as *Flammulina* and *Lentinus* were cultivated later in a similar manner, but the greatest advance in mushroom cultivation occurred in France around 1600 A.D. when *Agaricus* (champignon or button mushroom) was grown upon a composted substrate. In the years since World War II, there has been a consistent increase in mushroom production which greatly accelerated in the period from 1986 to 1989-90. A 74.4% increase was recorded during this latter period and total world production in 1989-90 amounted to 3.79 million metric tons valued at about US $7.5 billion (Table 2, Chang & Miles, 1991).

Table 2. Comparison of 1986 and 1989/90 world production of cultivated edible mushrooms.

Unit: (metric ton x 1000)

Species	Common Name	1986 Fresh	1986 % wt.	1989/90 Fresh	1989/90 % wt.	Increase
Agaricus bisporus bitorquis	Button mushroom	1,215	55.8	1,446	38.1	19.0
Lentinus edodes	Shiitake or oak mushroom	320	14.7	402	10.6	25.6
Volvariella volvacea	Straw mushroom or Chinese mushroom	178	8.2	207	5.5	16.3
Pleurotus spp.	Oyster mushrooms	169	7.8	909	24.0	437.9
Auricularia spp.	Wood-ear	119	5.5	400	10.5	236.1
Flammulina velutipes	Winter mushroom	100	4.6	143	3.8	43.0
Tremella fuciformis	White jelly fungus or "Silver Ear"	40	1.8	105	2.8	162.5
Pholiota nameko	"Nameko" or viscid mushroom	25	1.1	53	1.4	112.0
Hericium erinaceus	Monkey head mushroom or hedgehog fungus	---	--	90	2.4	
Hypsizigus marmoreus	Shimeji	--	--	22	0.6	
Grifola frondosus	Sitting mushroom or limuo, maitake	--	--	7	0.2	
Others		10	0.5	10	0.3	
Total		**2,176**	**100.0**	**3,794**	**100.2**	**74.4**

Source: Chang & Miles (1991)

In the Western world, *Agaricus* has remained the most extensively cultivated mushroom in quantitative terms and in the United States in 1990-91 still exceeded 99% of total U.S. mushroom production. However, mushrooms long popular in Asia (e.g. *Lentinus),* and produced there in large quantities, are now making inroads into Western markets.

Nutrition

Although mushrooms have long been appreciated for their desirable gastronomic properties of flavour and texture, and some for their medicinal and/or tonic attributes, they are only recently recognized as a food of high nutritional quality. Of particular significance, especially to regions with populations whose diet is commonly deficient in protein, is the protein content of mushrooms. It is now known that this is relatively high (19-35% on a dry weight basis) as compared to 7.3% in rice, 13.2% in wheat, 39.1% in soybean, and 25.2% in milk. Therefore, although mushrooms rank below most animal meats in crude protein content, they compare very favourably with most other foods (Crisan & Sands, 1978; Li & Chang, 1982a). With respect to essential amino acid indices, amino acid scores and nutritional indices, the overall nutritive value of high grade mushrooms almost equals that of milk (Table 3). Proteins of mushrooms contain all the essential amino acids and are especially rich in lysine and leucine which are lacking in most staple cereal foods. Furthermore, mushrooms contain a high proportion of unsaturated fatty acids, are a good source of several vitamins, minerals, fibre and other health promoting sub-

Table 3. Comparison of nutritive value of mushrooms with various foods.

Essential amino acid indexes		Amino acid scores		Nutritional indexes	
100	pork; chicken; beef	100	pork	59	chicken
99	milk	98	chicken; beef	43	beef
98	mushrooms (high)	91	milk	35	pork
96	*Volvariella diplasia*	89	mushrooms (high)	31	pork
91	potatoes; kidney beans	71	*V. diplasia*	28	mushroom (high)
	Pleurotus ostreatus	63	cabbage	27	*V. diplasia*
88	corn	59	potatoes	26	spinach
87	*Agaricus bisporus*		*P. ostreatus*	25	milk
86	cucumbers	53	peanuts	22	*A. bisporus*
79	peanuts	50	corn	21	kidney beans
76	spinach; soybeans	46	kidney beans	20	peanuts
74	*Lentinus edodes*	42	cucumbers	17	cabbage
72	mushrooms	40	*L. edodes*	15	*P. ostreatus*
69	turnips	33	turnips	14	cucumbers
53	carrots	32	mushrooms (low)	11	corn
44	tomatoes	31	carrots	10	turnips
		28	spinach	9	potatoes
		23	soybeans	8	potatoes
		18	tomatoes	6	carrots
				5	mushrooms (low)

Ranking based on essential amino acid indexes, amino acid scores and nutritional indexes as calculated against the FAO reference protein pattern. Values for mushrooms represent the mean of the three highest values (high) and the three lowest values (low). Source: Crisan & Sands (1978) and Li & Chang (1982a).

stances, and are low in calories, sodium, fat and cholesterol (Chang & Miles, 1989; Breene, 1990; Jong & Birmingham, 1990; Buswell & Chang, 1992). In addition, the nucleic acid content of mushrooms is not high enough to limit their daily use as a vegetable (Li & Chang, 1982b). Another compelling advantage of mushroom protein is that it can be produced with greater biological efficiency than proteins from animal sources. It is true that, in some highly industrialized countries, cultivation of the *Agaricus* mushroom is a highly sophisticated operation requiring a sizable capital outlay for controlled environment facilities. However, production of other mushroom species requires relatively little in terms of large-scale equipment, facilities, capital and land, and the mushrooms themselves often have less complicated demands in terms of processing.

Medicinal and tonic properties of mushrooms

Although mushrooms have traditionally been used in China and Japan for their medicinal and tonic properties, these aspects of mushrooms remain largely unexploited. However, there has been a recent upsurge in interest in traditional remedies for the treatment of various physiological disorders and numerous biologically active compounds have been reported in mushrooms as a result. Lists of pharmaceutical products developed from mushrooms in Japan (Table 4) and their

Table 4. Pharmaceuticals developed from mushrooms in Japan.

Name	Krestin	Lentinan	Schizophyllan
Abbreviation	PSK/PSP		
Date for sale	May 1977	December 1985	April 1986
Mushroom species	*Coriolus versicolor* (mycelium)	*Lentinus edodes* (fruiting body)	*Schizophyllum commune*
Polysaccharide	Beta-1,6 branch; Beta-1,3; Beta-1,4 main chain	Beta-1,6 branch; Beta-1,3 main chain	Beta-1,6 branch; Beta-1,3 main chain
Molecular weight	ca. 100,000	ca. 500,000	ca. 450,000
[alpha]$_D$	--	+ 14~22°C (NaOH)	+ 18~24°C (H_2O)
Products	1 g/package	1 mg/vial	1 g/2ml bottle
Administration	Oral	Injection	Injection
Indication	Cancer of digestive system, breast cancer, pulmonary cancer	Gastric cancer	Cervical cancer
1985 sale value	$556M	$85M	$128M

Source: Pai et al. 1990

active components (Table 5) have been compiled recently by Pai *et al.* (1990). *L. edodes* (shiitake) contains a drug Lentinan which has been shown to stimulate production of a polypeptide, Interleukin 1. This compound has metabolic, hormonal, immunologic and haematologic activities. Lentinan induces prostaglandin formation, stimulates T-lymphocytes, which are depressed in cancer states and also in AIDS, and has an anti-viral effect through the production on Interferon gamma. The reported effect on longevity is probably due to these anti-cancer, anti-viral and anti-inflammatory functions. Medical evidence has shown that ingestion of shiitake also prevents blood platelets from adhering to each other and thereby reducing the formation of clots. Cosmetic products and some healthy beverages have also been produced in China from mushrooms of *Ganoderma*.

Table 5. Pharmaceutical components of mushroom species.

Pharmacodynamic	Component	Species
1. Antibacterial effect	Hirsutic acid	Many species
2. Antibiotic	E-beta-methoxyacrylate	*Oudemanxiella radicata*
3. Antiviral effect	Protein, Polysaccharide	*Lentinus edodes* and *Polyporaceae*
4. Cardiac tonic	Volvatoxin, Flammutoxin	*Volvariella*
5. Decrease cholesterol	Eritadenine	*Collybia velutipes*
6. Decrease level of blood glycogen	Peptide glucan, Ganoderan	*Ganoderma lucidum*
7. Decrease blood pressure	Triterpene	*Ganoderma lucidum*
8. Antithrombus	5'-AMP, 5'-GMP	*Psalliota hortensis*
9. Inhibition of PHA	r-GHP	*Psalliota hortensis, Lentinus edodes*
10. Antitumor	Beta-glucan RNA complex	Many species, *Hypsizygus marmoreus (Lyophyllum shimeji)*
11. Increase secretion of bile	Armillarisia A	*Armillariella tabescens*
12. Analgesic, sedative effect	Marasmic acid	*Marasmius androsaceus*

Source: Pai et al. 1990

Bioconversion of waste materials

Mushrooms are a special group of fungi. Unlike green plants, fungi lack chlorophyll and consequently cannot use solar energy to manufacture their own food. However, mushrooms do produce a wide range of extracellular enzymes that enable them to degrade complex organic substrates into soluble substances which can then be absorbed by the mushroom for its own nutrition (Wood & Fermor, 1982; Wood, 1984). This absorptive nutrition is a characteristic of mushrooms. Many of the complex substrates suitable for mushroom cultivation are waste products, huge quantities of which are generated annually through the activities of agricultural, forest and food processing industries. Examples include cereal straws, bagasse, banana leaves, coffee grounds, sawdust, and cotton wastes from textile factories (Chang, 1991). These agricultural and industrial waste products are found in abundance in those developing regions of the world with economies which are still basically agricultural. Currently, much of this material is either burnt, shredded and/or composted for landfill or improvement of soil quality, even though these wastes constitute a valuable resource for human food production. Edible mushroom production also represents an attractive method of upgrading lignocellulosic wastes. The nutritional quality of the material remaining after mushroom harvesting may be improved sufficiently for use as an animal feedstock. Alternatively, the residue can be used as a soil fertilizer and conditioner, thereby increasing production of other agricultural and horticultural crops. Thus, edible mushroom cultivation represents one of the most economically viable processes for the bioconversion of certain types of organic wastes and although physical and chemical technologies may, in some cases, play important associated roles, biotechnical approaches are essential for the emergence of practical conversion processes which can be applied to situations in developing countries.

Cottage industry

Many people have been attracted to mushroom cultivation precisely for the reasons described above. The use of cheap, readily available raw materials, and reduced demands on land space compared to other agricultural crops are attractive features of mushroom cultivation particularly where large-scale capital-intensive operations are inappropriate. Also, in an environmentally conscious society, the bioconversion of potential pollutants into a food for human consumption is not to be dismissed lightly. There is no doubt that mushroom cultivation technology, if properly promoted and developed, can be translated into thriving 'cottage industries' which will make important contributions to the nutrition and economic welfare of many people, particularly in developing countries.

It is interesting to consider the recent cultivation history of *Agaricus bisporus* as a good reference for the cultivation of other edible mushrooms. Fifty years ago, the yield of *Agaricus* was less than 5 kg/m^2 (1 lb/sq ft) in more than twelve picking weeks. The picking was done by hand. Today, the top yields of this mushroom can reach 50 kg/m^2 (5.3 lb/sq ft) in four weeks and the harvesting can be done using a cutting machine. However, mushroom cultivation and the successful achievement of a profitable mushroom farm requires an understanding of certain scientific principles and practical experience in mushroom technology. Commencement of mushroom production on a cottage industry scale can be undertaken by villagers once they have acquired some limited technical knowledge. Once the basic skills are mastered, further progress will follow as a result of the natural ingenuity of the people and the adoption of modifications befitting the local environment. However, for a successful cottage mushroom industry, some cooperative operations are essential; e.g. collective preparation of high quality compost for mushroom production, and centralized processing systems to ensure high quality mushrooms for marketing.

Mushroom science

Although the cultivation of edible mushrooms dates back many centuries, research in this field is still relatively adolescent and limited to certain scientific institutes in developed countries. Only in recent years have research and extension laboratories been established in a few developing countries through the aid of national and international agencies. Mushroom science embodies the principles of microbiology, environmental technology, and solid state fermentation in the conversion of domestic, agricultural, and industrial inorganic waste materials into food for humans. The technology for mushroom cultivation can be primitive, as in rural farming of *Volvariella* and *Pleurotus* mushrooms. It can also be highly industrialized, as in *Agaricus* and *Lentinus* production in urban areas, in which advanced technology and equipment are used. Biological efficiency, i.e. the yield of fresh mushrooms in proportion to the weight of compost at spawning, can reach 100% in experimental tests, with 40-60% as a good average value per crop.

The activities centered around mushroom studies have achieved global dimensions and hold many long term implications. These include the conservation of mushroom germplasm as part of the need for wider protection of the Earth's biological diversity which has emerged as a matter of serious international concern. However, progress in mushroom cultivation and the development of the industry are dependent upon the collective efforts of scientists from both industrialized and developing countries. To achieve the most effective collaboration, an international research and training centre for mushroom studies should be established

Mushroom biology

Mycology is the science concerned with fungi, of which around 80,000 species have been described. Fungi are important to humans for a variety of reasons. They are the principle causal agents of plant diseases as well as some significant diseases affecting humans. Through their fermentative activities, fungi are major fabricators of several important products such as ethyl alcohol, citric acid, and the antibiotic, penicillin. With annual production valued at US $7.5 billion, the edible mushroom is certainly not to be ignored. Several terms for this important branch of mycology have been used in the past, each with its own merit. However, if the matter of definitions is considered more deeply, it appears that there is a place for a new term. The new term is mushroom biology. Mushroom biology is the new discipline concerned with the scientific study of mushrooms (Chang & Miles, 1992). The term mushroom science already exists, but is restrictive in that it has been defined as the discipline concerned with the principles and practice of mushroom cultivation. Confined solely to mushroom cultivation, mushroom science is only one aspect, albeit a significant one, of mushroom biology. Mushroom biology takes in not only cultivation but embraces all aspects of mushrooms including taxonomy, development, nutrition, physiology, genetics, pathology, medicinal and tonic attributes, edibility, toxicity, etc.

Professor S.T. Chang, Department of Biology, The Chinese University of Hong Kong, and his colleagues and collaborators have combined their own research data with available information on mushroom biology and mushroom cultivation collected from numerous sources worldwide to produced several volumes in the field. These books, which are listed below, have formed a basis for further academic research, technical extension and marketing promotion of mushrooms.

(1) *The Chinese Mushroom (Volvariella volvacea): Morphology, Cytology, Genetics, Nutrition and Cultivation.* The Chinese University Press, Hong Kong.
(2) *The Biology and Cultivation of Edible Mushrooms.* Academic Press, New York & London.
(3) *Tropical Mushrooms: Biological Nature and Cultivation Methods.* The Chinese University Press, Hong Kong.
(4) *Edible Mushrooms and Their Cultivation.* CRC Press, Inc., Boca Raton, Florida.
(5) *Technical Guidelines for Mushroom Growing in the Tropics.* Food and Agriculture Organization, Rome.
(6) *Culture Collection and Breeding of Edible Mushrooms.* Gordon & Breach, Inc., Philadelphia.

Edible mushroom species already under cultivation

As mentioned earlier, out of 80,000 described species of fungi, there are about 10,000 species of fleshy macrofungi. About 2,000 species from more than 30 genera are regarded as edible (Table 6). Unfortunately, some other mushrooms are very poisonous and there are no general guidelines for distinguishing between the poisonous and edible species. Only about 80 of the "edible" types are grown experimentally and about half of these have been cultivated economically. Approximately 20 edible mushrooms have been grown commercially but only about 5 or 6 *(Agaricus, Lentinus, Pleurotus, Auricularia, Volvariella, Flammulina)* are produced extensively (i.e. in several countries) on an industrial scale (Table 7). In general, the oriental countries, China, Japan and Korea, grow and consume more varieties of mushrooms than the western countries. However, in recent years, the production of what are referred to as "specialty mushrooms," mainly *Lentinus edodes,* and *Pleurotus* spp., have increased rapidly in western countries (Chang & Miles, 1991).

The important cultivated edible mushrooms are *Auricularia auricula, A. polytricha, A. fuscosuccinea, Tremella fuciformis, Hericium erinaceus, Lentinus edodes, Pleurotus ostreatus, Flammulina velutipes, Agrocybe cylindracea, Volvariella volvacea, bombycina, Kuehneromyces nameko, Pholiota adiposa, Dictyophora indusiata, D. duplicata, Hypsizigus marmoreus, Grifola frondosas,* etc. Many wild mushrooms have been used by the natives of various parts of the world. The important ones are: *Morchella esculeta, M. conica, M. angusticeps, M. crassipes, Cantharellus cibarius, Boletus edulis, Suillus luteus, Paxillus involutus, Gophidius viscidus, Lactarius deliciosus, Russual delica, R. virescens, Hohenbuehelia serotina, Calocybe gamblsa, Leucopaxillus giganteus, Aemillariella mellea, A. tabescens, Trucholma matsutake, T. mongolicum, T. terreusm, T. flavorireus, Agaricus campestris, A. arvensis, Naematoloma sublateritum,* etc. The important pharmaceutical ones are: *Poria cocos, Tremella fuciformis, Polyporus myclitae, Shiraia bambusicola, Cordycepts sinensis, C. sobolifera, Polyporus umbellatus, C. oriolusvesicolor, Gloeostereum incarnatum, Armillariella mellea, A. abescens,*

Table 6. Genera of prime edible mushrooms.

Genera of Prime Edible Mushrooms	
Basidiomycetes	
Agaricus bisporus	*Lentinus edodes*
Amanita caesarea	*Lepista nuda*
Armillaria mellea	*Lyophyllum decastes*
Auricularia polytricha	*Marasmius oreades*
Boletus edulis	*Pleurotus ostreatus*
Cantharellus cibarius	*Pholiota nameko*
Calvatia gigantea	*Polyporus frondosus* (*Grifola frondosus*)
Clitocybe geotropa	*Russula aureta*
Coprinus comatus	*Stropharia rugoso-annulata*
Cortinarius spp.	*Termitomyces albuminosus*
Dictyophora indusiata	*Tremella fuciformis*
Flammulina velutipes	*Tricholoma matsutake*
Gloeostereum incarnatum	*Volvariella volvacea*
Hericium erinaceus	
Lactarius deliciosus	
Ascomycetes	
Morchella esculenta	*Tuber melanosporum*

Table 7. Species of commercially cultivated edible mushrooms

Species of Commercially Cultivated Edible Mushrooms	
*Agaricus bisporus**	*Flammulina velutipes**
*Agaricus bitorquis**	*Lentinus edodes**
Auricularia auricula	*Lyophyllum ulmarium*
Auricularia polytricha	*Pholiota nameko*
Auricularia fuscosuccinea	*Pleurotus ostreatus**
Dictyophora indusiata	*Pleurotus sajor-caju*
Dictyophora duplicata	*Pleurotus cystidiosus*
Gloeostereum incarn	*Pleurotus cornucopiae*
Grifola frondosus	*Pleurotus florida*
Hericium erinaceus	*Stropharia rugoso-annulata*
Hypsizygus marmoreus (=*Pleurotus elongatipes* and = *Lyophyllum shimeji*)	*Tremella fuciformis*
	*Volvariella volvacea**

*Species produced on an industrial scale.

Hericium erinaceus, Calvatia gigantiea, C. lilacina, Myconastrum corium, Lycoperdon perlatum, L. pyriformis, etc.

Climatic conditions

Mushrooms are produced in both the more-developed and the less-developed countries under a wide range of conditions ranging from outdoor cultivation in relatively primitive facilities to indoor cultivation in insulated, vapourproof buildings fitted with highly sophisticated computerized environment control systems.

In general, there are two distinguishing phases in the growth and development of a cultivated mushroom in its compost. These are the vegetative stage and the reproductive stage. In practice, the former refers to the spawn running phase and the latter to the fructification phase. Martin and Demain (1978) and Esser (1980) used "growth phase" and "reproductive phase" to describe the transition from mycelial growth to the formation of a specific morphogenetic structure in many fungi in the fermentation industry.

After planting the spawn onto a prepared compost, the mushroom mycelium will grow from the spawn into the compost and colonize the substrate. During the period of mycelial colonization, the mycelium secretes enzymes into the substrate food base, some of which is broken down to more simple, soluble organic compounds which can be transported into the hyphae where they may accumulate for the subsequent metabolic uses of the fungus. After a certain period of growth, the mycelia may achieve interconnections among themselves and interwindment with the substrate. The former facilitates the translocation of nutrients while the latter provides a stronger physical support for fruiting body formation. At this state of development the mycelium may be said to have become "established." This means that the mycelium is ready to enter the reproductive stage if the environmental "triggers" for fructification are provided.

The transition from vegetative stage to the stage of fruiting body formation in mushrooms is controlled by environmental factors. The optimum temperature for fruiting is generally lower than the optimum temperature for mycelial growth (Kurtzman, 1979). The temperature necessary for vegetative growth and for fruiting must be considered in selection of an acceptable mushroom (Tables 8 and 9), and it should be pointed out that strains or dikaryotic stocks of a species may differ in their required temperature ranges and optimal values so that even within a single species, selections can be made. Certainly, CO_2, light and other environmental factors can act as a "trigger" for fructification in some mushrooms. However, if the optimum growth conditions are known for a particular mushroom species and climatic conditions are known for a particular region, matching the two is feasible. Furthermore, by using appropriate breeding techniques, it is possible to extend the range of climatic conditions under which any one mushroom species will grow. In most cases, however, some sort of climatic control will be required to optimize growth and production rates and to minimize exposure to undesirable weather.

Table 8. Temperature range, substrate type, production-cycle time, and approximate yield of edible mushrooms from nonaxenic culture methods.

Species	Substrate	Temperature range		Production-cycle time	Yield[a]
		Mycelial growth	Fruiting		
Little or no pretreatment					
Lentinus edodes	Wood logs (outdoors, sometimes protected)	5-35 (24)[b]	6-25 (15) autumn (10) winter (20) spring	3-6yr spring/autumn	40
Auricularia auricula	Wood logs (outdoors, sometimes protected)	15-34 (28)	15-28 (22-25)	2-5 yr spring/autumn	2-12
Auricularia polytricha	Wood logs (outdoors, sometimes protected)	10-36 (20-34)	15-28 (24-27)	1-2 yr	20-40
Tremella fuciformis	Wood logs	5-38 (25)	20-28 (20-24)	3-6 yr 7 months/yr	10-30
Some pretreatment					
Volvariella volvacea	(1) Rice straw (outdoor)	15-45 (32-35)	22-38 (28-32)	4-6 weeks	6-10
	(2) Cottonwaste, rice straw (indoor)	15-45 (32-35)	22-38 (28-32)	2-3weeks	30-45
Pleurotus sajor-caju	(1) Pasteurized cereal straw (indoor)	14-32 (25-27)	10-26 (19-21)	4-10 weeks	80-100 or more
	(2) Fermented cereal	14-32 (25-27)	10-26 (19-21)	4-10 weeks	80-100 or more
Long composting process					
Agaricus bisporus	Composted cereal straw/animal manure mixtures	3-32 (22-25)	9-22 (15-17)	14-16 weeks	65-80
Agaricus bitorquis	As above	3-35 (18-30)	18-25 (22-24)	14-16 weeks	40-65

[a]kg Fresh weight/kg d.m.
[b]Figures within parentheses are optimal values.
Source: Smith, *et al.* (1988).

Table 9. Temperature range, substrate type, production-cycle time, and approximate yield of edible mushrooms from axenic culture methods.

Species	Substrate	Temperature range		Production-cycle time	Yield[a]
		Mycelial growth	Fruiting		
Flammulina velutipes	Sterilized sawdust, rice bran mixtures (polypropylene bottles)	3-34 (18-25)[b]	6-38 (8-12)	12-20 weeks	70-100
Lentinus edodes	As above (polyethylene bags)	5-35 (24)	6-25 (15) autumn (10) winter (20) spring	3-6 months	60-100
Auricularia auricula	As above (polythene bags)	15-34 (28)	15-28 (22-25)	8-10 weeks	20-25
Auriculana polytricha	As above (polythene bags)	10-36 (20-34)	15-28 (24-27)	6-8 weeks	70-85
Tremella fuciformis	As above (polythene bags)	5-38 (25)	20-28 (20-24)	6-8 weeks	80-100

[a]kg Fresh weight/100 kg d.m.
[b]Figures within parentheses are optimal values
Source: Smith, *et al.* (1988).

An examination of the history of the cultivation of *Agaricus bisporus* shows that, in many counties, cultivation has become virtually independent of external climatic conditions. Progress towards similar independence has also been achieved for other cultivated mushrooms and it is likely that the trend towards more precise climatic control will continue.

Growth substrates for mushroom cultivation

Mushrooms are grown, not directly on soil as are other crops, but on organic substrates, either untreated or composted. These substrates are mostly waste materials from the agricultural, forest and foods processing industries and may also include textile factory wastes. By using these materials for mushroom cultivation, these otherwise negative-value by-products can be recycled to produce additional food for human consumption. In the process, adverse environmental impacts may be avoided and waste disposal problems reduced or even eliminated. Examples of such materials include cereal straws, corn cobs, sawdust, bagasse, wood pulp, cotton waste, oil palm waste, banana leaves, poultry wastes, coconut husks, tree bark and leaves. Using appropriate technology, it should be possible to develop combinations of edible mushroom species and organic waste substrate suitable for any raw material which is readily available in a particular region. An additional benefit is that the spent compost remaining after the mushrooms have been harvested may also serve as animal feedstock, soil conditioner or fertilizer.

Acceptability of product to local end-user

One of the most important factors to take into account when determining the suitability of a particular mushroom species for cultivation in less developed countries is the acceptability of the product to the intended market. Any food product must be acceptable to the indigenous population, either traditionally or through commercial promotion, otherwise there is no market value. If the mushroom is to be marketed fresh, it must be a species which is acceptable on the basis of its organoleptic qualities to the people in the area where it is cultivated. Acceptability can be determined for a previously cultivated species by examination of import records, if available, or by testing for market acceptability with fresh mushrooms imported for that purpose. The selling price, which will be dependent upon production costs, will also need to be within the purchaser's range.

Cultivation methods and facilities

Mushroom cultivation can be a relatively primitive type of farming or a highly sophisticated agricultural activity requiring a sizeable capital outlay for mechanized equipment. The straw mushroom, *Volvariella volvacea,* is commonly grown in Southeast Asian countries on small, family-type farms. In contrast, cultivation of the *Agaricus* mushroom may be highly industrialized with a few farms producing a disproportionally large percentage of a country's output as is the case in many Western nations.

Although simple in concept, there are various intricacies associated with the process of mushroom cultivation which must be understood for the enterprise to be successful. In every case, the ultimate aim is to obtain the maximum yield from a given surface area per period of time by the use of high yielding strains, by shortening the cropping period, or by increasing the number of high yielding flushes. To achieve maximum yield requires an understanding of substrate materials and their preparation, appropriate control of physical, chemical and biological parameters (e.g. moisture content, pH, temperature, competitive microflora), and proper management of mushroom beds, including mushroom pest and disease control.

The major phases of mushroom cultivation are shown in Table 10 and are: (a) selection of an acceptable mushroom, (b) requirement for the selection of a fruiting culture, (c) development of spawn, (d) preparation of compost, (e) mycelial (spawn) running, and (f) mushroom development.

(a) Before any decision to cultivate a particular mushroom is made, it is important to determine if that species possesses organoleptic qualities acceptable (either traditionally or through commercial promotion) to the indigenous population and/or an export market, if suitable substrates for cultivation are plentiful, and if environmental requirements for growth and fruiting can be met without excessively costly systems of mechanical control. There is considerable variation among edible mushroom species in the temperatures suitable for vegetative growth (spawn running or mycelial running) and fruit body development.

Table 10. Major phases of mushroom cultivation.

Major Phases	Main Points to Consider
Selection of an acceptable mushroom	Location Climate Raw materials Acceptability
↓	
Selection of a fruiting culture	Tissue culture Spore culture (a) without mating for homothallic species (b) mating with compatible isolates for heterothallic species Mixed culture Preservation
↓	
Development of spawn	Substrate Vigorous growth Free of contamination Avoid use of senescent and degenerate spawn Good survival in storage
↓	
Spawning	
↓	
Spawn running → Fructification (mushroom development)	Establishment of mycelium Environmental requirements (a) temperature (b) light (c) aeration (O_2, CO_2) (d) pH (e) moisture Casing Watering and care
↑	
Composting	
↑	
Preparation of compost	Concept of composting Microbial activity Softening of substrate for ease of colonization Physical characteristics Chemical components Aeration Water content

Source: Chang (1991)

(b) A "fruiting culture" is defined as a culture with the genetic capacity to form fruiting bodies under suitable growth conditions. The stock culture which is selected should be acceptable in terms of yield, flavour, texture, fruiting time, etc.

(c) A medium through which the mycelium of a fruiting culture has grown and which serves as the inoculum or "seed" for the substrate in mushroom cultivation is called the mushroom spawn. Failure to achieve a satisfactory harvest may often be traced to unsatisfactory spawn. The potential of the spawn is ultimately set by the genetic constitution of the fruiting culture used in its manufacture. Ideal environmental conditions and management cannot overcome the limitations of a genetically inferior stock used for spawn production. Although the mushroom stock is of prime importance in determining the merits of a spawn, consideration must also be given to the nature of the substrate material used in spawn manufacture. This influences the rate of vegetative growth in the spawn substrate and also the rate of mycelial growth and filling of the beds following inoculation (spawn running). Some of the substrates used in spawn production include various grains (rye, wheat, sorghum), rice straw cuttings, cotton waste, rice hulls and cotton seed hulls. Availability and cost of the spawn substrate are also important. Some other obvious features of a good spawn include freedom from contamination, vigorous growth, and good survival in storage.

(d) While a sterile substrate free from all competitive microorganisms is the ideal medium for cultivating edible mushrooms, systems involving such strict hygiene are generally too costly and impractical to operate on a large scale. However, substrates for cultivating edible mushrooms normally require varying degrees of pretreatment in order to promote growth of the mushroom mycelium to the practical exclusion of other microorganisms. To accomplish this, certain chemical and physical qualities must be built into the substrate. Some edible species *(Lentinus edodes, Pholiota, Tremella)* can utilise lignocellulosic wastes, e.g. wood, with little or no pretreatment; others *(Volvariella volvacea, Pleurotus* spp.) can colonize plant material, e.g. cereal straws, after some composting, physical and/or chemical pretreatment, while *Agaricus bisporus* requires a lengthy controlled composting of cereal straws with manures or other nitrogen-rich additives. For *Flammulina* and some *Pleurotus* species, sterile substrates are prepared by autoclaving sawdust/rice bran mixtures or straw. The substrate must-be rich in essential nutrients in forms which are readily available to the mushroom and be free of toxic substances which inhibit growth of the spawn or may be taken up (and perhaps concentrated) by the fruit body. Moisture content, pH and good gaseous exchange between the substrate and the surrounding environment are important physical factors to consider.

In practice, composting is accomplished by piling up the substrates for a period during which various changes take place so that the composted substrate is quite different from the starting material. A substrate consisting of agricultural and chemical materials other than animal manure, after composting is called a "synthetic compost." Synthetic composts have been devised using numerous formulations of just about every type of agricultural waste product and residue. All these materials consist mainly of cellulose, hemicellulose and lignin. Some bacteria readily attack the polysaccharide components cellulose and hemicellulose and, under suitable conditions, bring about their decomposition. However, the lignin component is more resistant to bacterial attack. The more readily degraded carbohydrates serve as a nutrient source for bacteria and fungi and levels diminish after composting as a consequence of the metabolic activities of the microorganisms in the compost. This makes the substrate less favourable for growth of these potential competitors to the growth of mushrooms. The metabolic activities of the microorganisms also have other effects:

(i) conversion of simple nitrogenous materials, such as nitrates and ammonia, into complex proteins, thereby increasing the protein content of the compost required for later growth of the mushroom mycelium, and

(ii) a drop in pH. Research on mushroom composts has been extensively reviewed (Nair, 1991).

Many of the principles of this complicated process of composting are recognized and general guidelines are available for the grower. In practice, modifications are necessary to meet the various local conditions encountered. These may be necessitated by availability of raw materials, the microflora in the composting area, and, especially important, the species of mushroom to be cultivated. The highly industrialized technology developed for the cultivation of *Agaricus* cannot be followed unmodified for the commercial production of the straw mushroom, *Volvanella volvacea.*

(e) Following composting, the substrate is placed in beds where it is generally pasteurized by steam to kill off potential competitive microorganisms. After the compost has cooled, the spawn may be broadcast over the bed surface and then pressed down firmly against the substrate to ensure good contact, or inserted 2-2.5 cm deep into the substrate. Spawn running (mycelial running) is the phase during which mycelium grows from the spawn and permeates the substrate. Good mycelial growth is essential for mushroom production and will depend on proper maintenance of the beds and mushroom house in terms of temperature, moisture content, humidity) and aeration.

(f) Under suitable environmental conditions, which may differ from those adopted for spawn running, primordia formation occurs followed by the production of fruiting bodies. The appearance of mushrooms normally occurs in rhythmic cycles called "flushes." Harvesting is carried out at different maturation stages depending upon the species and upon consumer preference and market value. Suitable temperature, humidity, and ventilation controls must be maintained during the cropping period since these factors will affect the number of flushes and the total yield obtained.

Concluding Remarks

As the population of the world continues to increase, so the amount of food and the level of medical care available to each individual, especially those living in less developed countries, decreases. Mushrooms, with their great variety of species, constitute a cost-effective means of supplementing the nutrition of the majority of humankind and of alleviating the suffering caused by certain kinds of illness. Furthermore, with proper training and supervision, mushroom cultivation can be introduced to the small farmer in different regions of the world as a means of generating income, thereby raising the economic and social status of the rural population in less developed countries as well as in industrial countries.

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THE MULTIPLE USES OF DEEP OCEAN WATER

Comments by
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At the Natural Energy Laboratory of Hawaii Authority at Keahole Point here in Kona (Figure 1) we are demonstrating multiple synergistic uses for deep ocean water pumped ashore in the tropics. As can be seen in Figure 2, the cold deep water can be used in conjunction with warm surface water for uses as diverse as energy conversion; freshwater production; industrial condensation, ice making and dehydration processes; air conditioning; aquaculture; and agriculture of temperate plants in the tropics.

Ocean thermal energy conversion (OTEC), in which electricity is produced from the temperature difference between surface and deep water, was the initial focus of the work at NELHA. We have demonstrated that OTEC works and have developed several important innovations which improve the reliability and reduce the capital cost of the process. OTEC is, however, an inherently large-scale technology, and sufficient funding has not been available to construct and operate the pilot plant which will be necessary to demonstrate the viability of commercial-size systems. OTEC has not yet, therefore, been shown to be competitive at current prices of oil. Many investigators, however, believe that the synergistic uses of OTEC's deep water might combine to make an economically viable system.

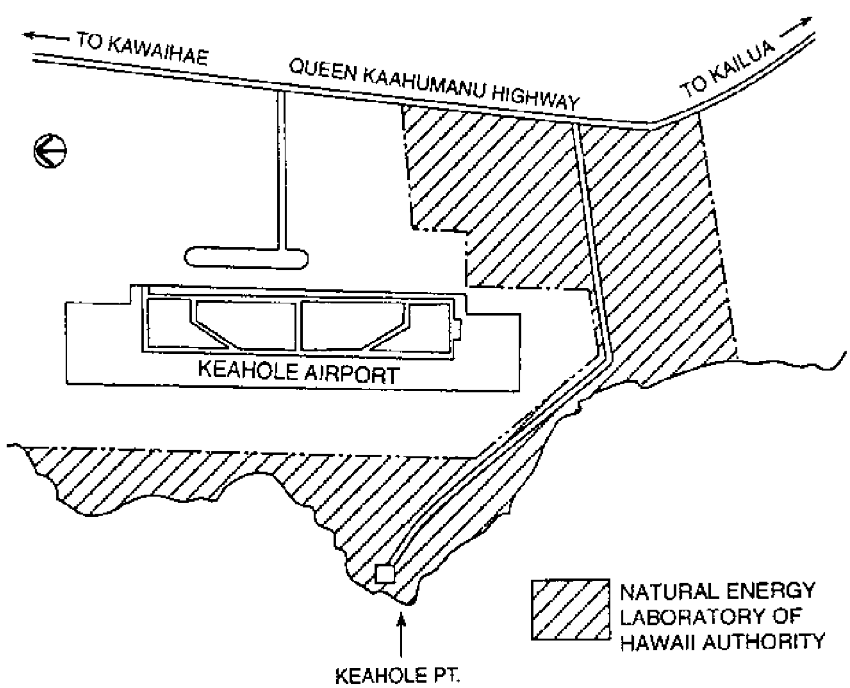

Figure 1. *A vicinity map of the Natural Energy Laboratory of Hawaii Authority on the Kona coast of the Big Island of Hawaii.*

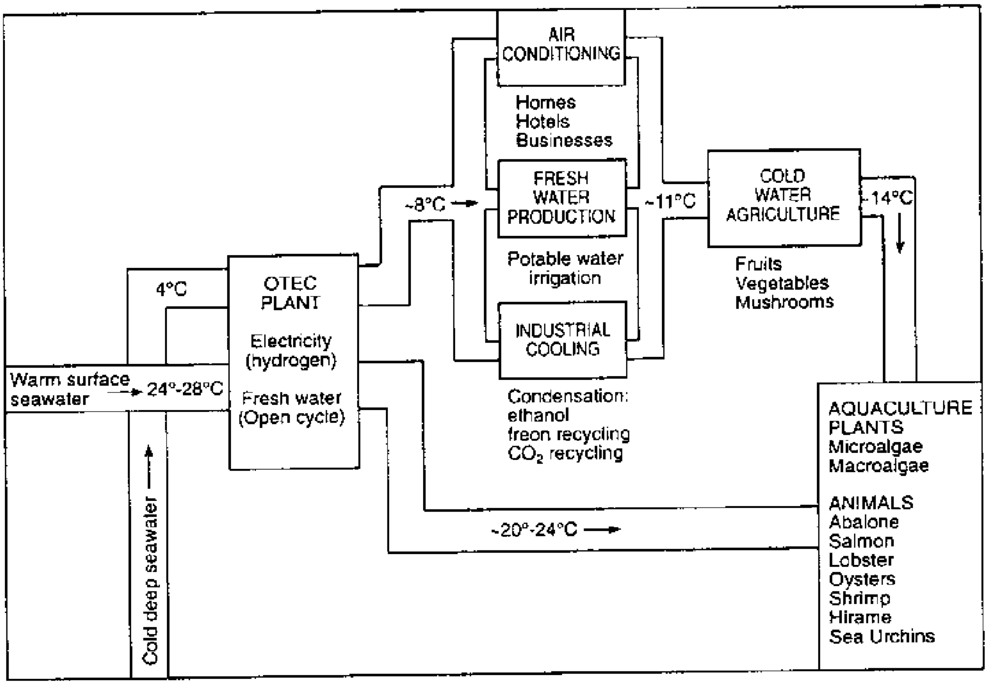

Figure 2. Schematic of an idealized integrated energy/aquaculture/agriculture system based on the use of cold deep seawater.

One of the most attractive uses of the cold seawater is for freshwater production. In its simplest form, potable water condenses from our humid tropical air onto heat exchanger plates chilled with the cold deep seawater. Experiments indicate that, at our average relative humidity of 70%, we can produce 5 gallons of fresh water for every 1000 gallons of 7°C seawater flowing through the system. Not only will this be enough water to meet all of the drinking, bathing and agricultural needs of the population, the removal of moisture has significant potential for improving climate by reducing the relative humidity downwind from the plant.

Several projects at NELHA are demonstrating uses of the deep seawater for condensation. Cyanotech Corporation, which produces *Spirulina* microalgae in large raceways for health food, has found that they can use the cold water to aid in the recovery of carbon dioxide from the exhaust of their propane fired drier. The CO_2 is injected into the raceways, promoting the growth of the *Spirulina* and saving the company thousands of dollars per month. Another company is developing the distillation of ethanol from sugar cane molasses using a solar boiler and a deep-seawater-cooled condenser. Yet another tenant is demonstrating the recycling of air conditioner freon by using the cold seawater in a system which distills out the oil contaminants which decrease the efficiency of used freon.

Direct use of the cold deep seawater for air conditioning has the potential to reduce electrical consumption by ten times as much as could be obtained from the same seawater in an OTEC system. For example, the new 55-inch diameter pipeline NELHA is designing will feed a 1 MW OTEC plant, but it can reduce air conditioning electricity consumption by about 10 MW. Studies indicate that this cooling could be distributed as far as 50 miles from the coastline, offering the potential of providing air conditioning to more than 50% of the U.S. population.

Other obvious uses for the cold deep water include dramatically reducing the energy requirements for refrigeration, ice making and other industrial cooling needs. Several firms are investigating the feasibility of refrigerated warehouses and ice factories using the cold seawater available at NELHA.

Aquaculture of many species of plants and animals has become the most visible and well-publicized activity at NELHA. The coldness of the deep water allows the growth here in the tropics of coldwater organisms such as salmon and abalone. Our tenants also take advantage of the year-round availability of large volumes of seawater at any temperature between 6°C and 24.5°C, obtained merely by twisting a valve to control mixing of surface and deep water. The deep water is also very rich in the dissolved inorganic nutrients necessary for plant growth and nearly devoid of pathogens, making it eminently suitable for culture of sensitive larvae. We'll see some examples of these aquaculture projects during our tour of the laboratory.

The subject of this workshop, dubbed COLD AG by Dr. Craven, is another of the exciting uses of cold deep seawater in the tropics. Workers at NELHA have already demonstrated prolific growth of strawberries, *Alstroemeria*, lettuces and asparagus—none of which would otherwise grow well in our subtropical climate. From what we are learning here today, this technology appears to have truly limitless possibilities.

The work we've done at NELHA has barely begun to scratch the surface of the potential uses of deep seawater. As John Craven likes to say, the greatest resource of the sea may well be the deep ocean water itself!

NOTES FROM DISCUSSION AND COMMENTARIES BY PARTICIPANTS

Facilitators: Dr. John P. Craven and Dr. Ellen Weaver

Editor's Note: The selections below were edited from a transcript of discussions by the "COLD AG" Workshop participants on the second day of the Workshop.

Co-Chair Ellen Weaver opened the discussion session by introducing Michael Harburg of White Rainbow who "is thinking about mushrooms and has just given me a proposal."

Mr. Harburg explained that his company's intention is to commercially cultivate edible and other mushrooms at Keahole Point (NELHA). This will be conducted in several phases; the first of which should take about 18-24 months. The primary goal of this research is development of cultivation methods in outdoor temporary structures that are unified by cold ag technique, thus creating a microclimate favored by fungi. Research will also be done in other areas including marketing, general mycological processes, energy and material cost associated with creating the microclimate for production of fungi. Among the assets recently contracted for purchase from another NELHA tenant is a thermal recovery system (boils freon and has a solar still). A highly efficient heat exchanger system will provide an inexpensive source of cool freshwater for climate control.

A small spawn production laboratory will be installed, including a laminar flow booth used in inoculation and autoclaves for sterilization. In the laboratory spawn will be propagated and selected for vigor and purity, spawn masters kept in jars, and mycelial logs will be incubated in a cool clean atmosphere. When the logs are totally covered with mycelia, they will be removed from the refrigerator and moved to an incubator. A suitable microclimate will be created using coldwater technique. Major costs will include sterilization of culture media, creating the micro-climate, and cooling the incubation room. NELHA offers this company, now in its research phase, very low cost, "unbeatable." Elsewhere, refrigeration costs a fortune while out there it costs pennies. In Hong Kong, where mushrooms are grown commercially, cooling coils are used to create the desired microclimate at great cost.

It was pointed out that there are mainland niches, e.g., Pennsylvania is one. A lot of culture houses are dug deep enough below ground so the temperature year round is kept fairly constant. Economically, since mushrooms don't travel well (limited to a 3-500 miles range) and have a shelf life of about 10 days, there is a niche for local source. Since cold is not an expense, the next most significant cost will be labor.

Automation can provide some relief, depending on the technology selected. John Craven, President of Common Heritage Corp. (CHC), pointed out that it doesn't have to be costly to automate if low tech such as "ropes, pulleys, weights" are used. Considering the use of energy, it turns out that a combination of cheap high tech and old fashioned low tech ought to be able to automate these processes rather economically.

CHC plans to build "microclimate towers" to grow crops such as strawberries and mushrooms as well and intends to use some form of automation to lower labor costs.

Cary Mitchell was asked to explain the minitron, similar in concept to the microclimate tower. Development of the minitron was started in the late 1970's as a research tool. In dealing with whole plants or canopies of plants as opposed to cells and tissues, researchers saw a need to be able to measure photosynthetic and respiratory gas exchange under the same conditions that the plants were growing under. Prior to that, photosynthesis scientists were growing plants in growth chambers or greenhouses, and then they would move them to a laboratory where they would set up special cuvettes.

Minitrons were essentially whole-plant or small-canopy gas exchange cuvettes with environmental control. They would be located right in a walk-in growth room so scientists could measure gas exchange characteristics of these canopies in the dark, in the light, and the same conditions they were grown under without any kind of disturbance whatsoever. They were made to be temperature-controlled so there would be a heating, cooling, and circulating heat exchanger system outside of it. Coolant was pumped through an automobile heat exchanger inside the minitron, and an internal fan system would circulate the air in a butterfly pattern to get uniformity of temperature and also gas composition.

Another nice feature of the minitron system was a computer-controlled mass-flow control system for injection of CO_2 to modify the gaseous environment around the shoot canopy. Also photosynthetic and transpiration rates could be monitored from the canopy under these modified controlled atmospheres. The minitrons were set up with the plant growing container where you had the option of growing the plant or plants hydroponically with separate aeration of rhizosphere, the root atmosphere separate from the shoot atmosphere, so you could have CO_2-enriched air coming in and flowing through the minitron through the canopy and then out to infrared gas analyzers. One could also throw a switch and switch over to root outlet atmosphere and then measure root respiration. So the minitron became a very valuable research tool and also could give statistical replication, very difficult to do in larger, more expensive growth chambers.

Minitrons are fairly economical; are made out of transparent plexiglas cylinders with lids. First generation were made 12 inches in diameter, the second generation 24 inches in diameter and same height or higher, then later were made 3 feet wide. They were made transparent so that PAR (photosynthetic active radiation) could be put in through the tops and also through the sides. Dr. Mitchell commented that they "have been wonderful for our CELLS program sponsored by NASA to define optimum environments for growing crops." The goal of CELLS is to get maximum growing activity in a minimum growing area per unit time. Crop productivity is measured as grams dry weight of biomass per square or per growing volume per day—gm/m^2/day. They have been able to take crops like leaf lettuce which produce in the field in California 2.5 gm/m^2 up to 67 gm/m^2 with 19-21 cropping days instead of (the) usual 55 -70. Their colleagues at Utah State University have taken cultivar wheat and cut production cycle from 120 to 65 days cropping cycle and over 60 gm/m^2 using similar techniques.

Dr. Mitchell noted that "it will be worthwhile for people at this conference to know we are working through NASA funding in a fledgling field called CEA: controlled environment agriculture."

Dr. Craven added that in carrying out R&D in these areas, cheap alternatives for factors of highest cost in each operation must be sought. For example, the use of very expensive lamps could be ruled out to create the different atmospheric conditions for roots, stem, crown of the plants. He added: "I want to keep stressing over and over again the economical advantage of cheap cool and hot at Keahole Point (NELHA). We have the functional knowledge to put these together to create controlled environments. We are also learning what environments we have to have to produce crops during their whole growth cycle, so we can begin to construct cheap towers and then let nature do its part. That is what my is program is dedicated toward. If we fail we will still make a lot of progress. We will apply the best knowledge that we have in the design."

Co-Chair Weaver noted that Dr. Glenn had a presentation to add to this...CEA topic about growing lettuce.

Dr. Glenn's group "spent a lot of years trying to develop CEA for coastal deserts and for deserts in general. Slides showed desert in Abu Dhabi and these are seawater basins, seawater is a cooling medium for *Salicornia*. Mainly what we grow out there are heat tolerant crops such as eggplant, tomato, cucumber. When I heard about this "Cold Ag" Workshop, I recalled the lettuce project in Tucson, Arizona, for the Kraft project niche market for year round production in desert environment...real life marketing ...sold these with roots on, a real nice marketing concept...Used an idea developed by Merle Jensen...nice process, recycle moisture."

In this project, Dr. Glenn explained that floating flats of lettuce were used in raceways filled with nutrient solution. Rubber liners were put over curbing to create the raceways and air stones used to aid circulation. "City water" was usedto create a large reservoir of water "which we were able to analyze once a month; added dry chemicals to maintain nutrient levels...the key to it was (having) a large reservoir of water."

In Tucson, Arizona, we had two acres of lettuce growing in raceways. The plants floated on styrofoam boards; turns out the stryrofoam boards served as insulation which helped maintain temperature in the raceways. Started with seedling operation; stocked seedlings in raceway at one end; they were moved forward by putting new sedlings in at one end, harvest size heads out at other end. Simple way of harvesting, not machines, but very automated, is automated like Craven said; push them down with a stick. (Craven noted, "That's automation.")

Dr. Glenn further explained an "interesting finding" on what made the lettuce grow. He showed a series of slides of graphs. The first graph showed the harvest weight of standard size heads at 42 days after seeding them. The second graph showed the radiation curve inside the greenhouse and a third curve showed the daytime and nighttime temperatures. There was an obvious correlation between radiation levels and harvest weight in one of the brightest climates—"not exactly like Keahole"—in the U.S.

He went on to explain, "We got a positive response to temperature right up to brightest days of the year." Although at first it looked like there was no response to temperature, "when we did a correlation matrix we could see there was a significant correlation with day temperature as well as with light levels. We analyzed the different crops, and found basically that the fall crops grown under cool temperatures responded differently than the spring crops. We were able to resolve the whole response to light and temperature by interaction of light and temperature, so up to the hottest day of the year and the brightest day of the year, this lettuce, which is supposed to be a cool weather crop, was responding positively. So that means that the highest production is going to be in the sunniest, warmest spot in world." He and his colleague put their data, representing the high end of the radiation curve, with literature values where lettuce is normally grown at the lower end.

In the graph used to illustrate this point, "The bottom (axis) is radiation levels going from zero up to about 500 cal/sq cm/day and the other axis days to harvest. So 'less days to harvest' is a higher growth rate." He summarized, "Over the whole range of environments around the world in which lettuce has been grown it responds positively to radiation. The Tucson data was really outside the range that had been reported in the literature for the normal growing climate."

A question was raised to clarify the upper radiation levels used and their equivalents in other units. 500 cal/cm sq./day could be roughly equilibrated to about 1500 to about 2000 microeinsteins per day or about three-quarters to full sunlight, "about 65% transmission through the greenhouse top."

Dr. Glenn explained some of the growing problems encountered, which is where the notion of "COLD AG" comes in to this project. "We found that we started to lose some of the varieties of lettuce to bolting which is when the flower head comes out, and they get real ugly and bitter. The very susceptible varieties started to bolt as early as February; by April and May nearly all of the heads were bolting, and it became a serious problem. It didn't really seem to be correlated with the minimum temperature since we could actually get those to go down as the year went along by evaporative cooling, and it didn't really seem to be so much related to the maximum air temperature. It seemed to be related to the nutrient solution heating up the root temperature.

"We were really desperate to try to keep growing this lettuce for the Kraft company through this summer period when the air temperatures were around 100°F. We looked at all kinds of things. We tried different varieties. We tried 'slow bolt' which bolted just as quick as everything else. This (slide) shows a normal butterhead type lettuce with a really well-developed heart to it. And we tried 'all the year round' (variety) which lasted about half the year for us. As you can see with the bolt-resistant varieties it just minimized the size of the center of the head. These varieties bolt just like all the rest but you can't see it for a while. And 'four seasons' (was) for us...like two and a half seasons."

He went on to explain, "We tried special cooling methods... a cooling tower built over a raceway...didn't work at all. It cut out the light level and didn't control the bolting. We tried fog nozzles and misting which is a real efficient cooling system and worked fine, but again it didn't control the physiology. Everything still bolted and went to seed."

A question was raised about the possibility of day length playing a role, but he replied that with lettuce daylength is part of the story, and increasing photoperiod potentiates the plant to bolt. However, by itself it will not cause bolting. "Our crops actually didn't start to bolt in a real serious way until July and August which is past the longest day in the year when the humidity in the air was so high we couldn't control the temperature, and the root temperatures especially got high."

Although it was noted that if left alone, the lettuce would eventually bolt anyway under long days, but they are usually harvested at saleable size long before the vegetative part of the cycle.

Glenn went on to explain that "we turned to putting ice in the nutrient solution, and we used 18000 pounds of ice per raceway. It worked out to about 15000 heads harvested per raceway. It all worked out to about a penny a head to ice the raceways, and the thing for this conference is we were only looking at a temperature of 22 or 23 degrees vs 26, 27 degrees. That was the difference between a saleable crop and one we had to throw away."

He showed some pictures of his as a comparison of two raceways, one which was chilled...to control the root temperature and one which wasn't. The average fresh weight per head was much higher in the chilled raceway. The percent saleable was almost 90% in the chilled raceway vs. only 61% in the unchilled. The percent undersized was much higher in unchilled raceways, so we got a growth response to chilling of roots, and we also controlled the bolting which was the most serious problem. "With the unchilled raceways we had to harvest them too early and undersized in order to get them before they bolted; where we could let them to go on to size."

Additionally, "we also had a problem with thermal dormancy of the seeds. When it got too warm the seeds wouldn't germinate. And that also turned out to be something we could control by pre-chilling the germinating trays after they were sown and hydrated. If we chilled them 24 hours, they would take any amount of heat after that and would go on to germinate. We also tried to grow spinach...(but) had a very serious disease problem, tipnia, which just wiped out all our spinach crops...it was also related to the temperature of the nutrient solution. At 20 degrees we basically didn't lose any plants at all to tipnia, but at 30 degrees we lost as much as 100% of our plants to what they call high temperature tipnia disease...We found a real nice correlation between the incidence of tipnia..and temperature. We could control disease and we could control physiology all with just a little application of cool around the roots of these plants."

Dr. Glenn added that "finally, once the cool is all gone from the seawater, the highest and best use of all of this seawater is to grow food crops for people in the desert. These are halophytes or crops that can be grown in seawater (e.g., *Salicornia bigolovlei* which is an oil seed crop).

He summarized the final outcome of the lettuce project. The project actually was technically successful but the cost of growing and packaging that butterhead lettuce and competing with iceberg lettuce which is what Americans eat...just wasn't feasible. Where this will really work is places where they like butterhead lettuce and greenhouse type lettuces, e.g., North Africa, European market, any place in the Middle East.

A question was raised regarding the difference between common lettuce crops, iceberg and butterhead, so Dr. Glen explained: "Iceberg is a much bigger head and requires more sunlight than you can normally get in a greenhouse environment. It just doesn't lend itself to greenhouses. Europeans now like the head type lettuce and are growing miniature iceberg that are made for their greenhouses. Maybe there's some potential there." Additionally, in the U.S. the typical iceberg head of lettuce is about a pound, really too big for one person consumption and often the consumer ends up "letting it rot in the refrigerator (whereas) these little greenhouse head lettuces generally do very well in the greenhouse business, good for the restaurant business."

Another question was raised, this time about why a greenhouse would have to be used in a warm climate such as Arizona. Glenn replied, "The answer is for cooling; it's just too hot to grow outdoors during the summertime. Actually, during six months of the year it's too hot. We use the greenhouse as a cooling structure." He noted that "greenhouses were developed to heat to grow crops in the off season when it's cold outside..."

Dr. Mitchell noted that an undesirable feature of greenhouses is that it cuts down incoming solar radiation by a third, yet that very feature contributes to cooling. Dr. Glenn agreed that "the greenhouse gives us a controlled airspace and we can apply cooling too; it's normally evaporative cooling..."

The need for alternatives to evaporative cooling methods (e.g., swamp coolers) was noted. In Arizona, swamp coolers work well most of the year,save about two months when the humidity rises. In Hawaii and other tropical humid environments, including coastal deserts, swamp coolers wouldn't work well, which, as one Workshop participant noted, "That's why OTEC is a real boon to these kind of projects." Cold deep ocean water, again, was noted as the key in low cost cooling methods that could be used for cooling in coastal environments.

The need for a greenhouse was questioned under the conditions described, since, as Dr. Glenn had shown, maximum leaf growth seemed to correlate to both maximum sunlight and relatively high temperature as long as the plant roots were kept cool. Dr. Glenn noted that the extremely high air temperatures without the greenhouse and the cooling pads would likely have been about 120°F and although the plants might have tolerated it, they don't prefer it. The data collected in the lettuce project were for a positive growth response to temperature interacting with light up to 31°C, still below 120°F(equivalent to about 41°C).

In addition to heat, another cause of leaf burn, calcium deficiency, was noted. This is referred to as 'tip burn,' apparently controlled very easily by applying calcium nitrate solution with a hand sprayer . It was explained that tip burn is a physiologic disorder of calcium deficiency brought on by outer leaves transpiring and drawing the calcium as it exchanges its way up the xylem cell walls to the outer leaves. It is particularly bad with head lettuces or lettuces that come up and shield the inner leaves and is a function of transpiration.

Dr. Glen made a final note about the down side of the method of growing lettuce hydroponically which was used in Tucson. Although it generally worked quite well, "there was one serious problem." The styrofoam boards, the cheap ones especially, purchase off the shelf, let the water wick up and wet the underside of the board surface allowing the wrapper leaves of the lettuce to rest in a small pool of water. This in turn resulted in "a terrible problem with *Fungus botritus*" which was finally controlled with chemicals, admittedly not the preferred way to deal with it. Therefore, anybody wishing to use this method commercially would have to find an impermeable top surface for the boards.

Dr. Mitchell elaborated on the value of containment in CEA, referring the Workshop participants to Blackman's Laws of limiting curves of limiting factors. By using a factor like cold seawater to moderate temperature, one can eliminate or remove the potential for a source of stress. However, it must be noted that that does not constitute full optimization of the production environment in containment structures like stratification towers or other forms of CEA. The potential exists for optimizing other factors as well, not the least of which is atmospheric composition, e.g., enriching the atmospheric carbon dioxide at some level concomitant with the amount of PAR—photosynthetically active radiation—to shorten crop/production cycle or enhance the yield rate at some stage in the growth cycle. He noted that "there's been substantial demonstration that both yield rates as well as the duration of crop production cycle can be significantly altered by incrementally optimizing environment with respect to light level and quality, temperature, humidity, and the atmospheric composition."

Although there are many classes or categories of crops compatible with each other in terms of CEA parameters, monocultures still might be preferred for optimization if the total environmental package for production is unique for the individual crops selected such as strawberries and mushrooms which have been discussed. Dr. Mitchell noted that in this regard, the workshop has "barely touched upon the rapidly developing science of controlled environment agriculture of which there have been many other programs and workshops already held."

A discussion to clarify the most efficient application of CO_2 enrichment led to the conclusion that generally, especially for green leafy crops, it would be appropriate only in the daytime when there's sunlight. However, for some crops such as potatoes, a 24-hour application of CO_2 enrichment would be preferred to enhance tuber growth through dark fixation.

Dr. Weaver, whose specialty is photosynthesis, further elaborated on the use of CO_2 enrichment. She explained that "CO_2 gets into plants through openings in the leaves called stomata which are exquisitely, beautifully controlled by the plant by a variety of factors: by the amount of water available, the amount of CO_2 available, the amount of light available, (and) obviously by the kind of plant. With instrumentation you can just sit and watch those little things opening and closing like blinds in response to every change."

A student of hers did a research project on the behavioral effects of CO_2 in two very closely related species and confirmed that high CO_2 levels generally make stomata close down. However, there is a lot of variety in different crops so one cannot just generalize and give plants "a huge slug of CO_2" and assume they'll do better. One would need to find out what combination of factors, including CO_2 levels and light intensity among others, is optimal for every set of conditions.

Dr. Weaver also reminded the group that oxygen, a waste product of photosynthesis, makes up 20% of our atmosphere. The less oxygen there is in CEA applications, the better net photosynthesis is in plants. If the oxygen content in a controlled atmosphere is reduced, that waste caused by respiration is also reduced. However, one cannot eliminate the oxygen entirely because then the plants would "go anaerobic and ferment and that's not good for them either." At levels perhaps as low as 1 or 2% (which would kill humans), the plants would likely thrive and grow far faster than normal (although less lignin woulud also be made).

Blue green algae don't need much oxygen to begin with; but she was surprised to find that when she accidentally grew them on nitrogen once instead of oxygen, they grew ten times as fast as they did on oxygen. No one had ever done that before, so it was quite a surprise. Oxygen is a poison for plants, so if we're going to look for optimum environments, she suggested substituting more nitrogen for an easy way to do it.

Dr. Weaver noted that there has been a lot of work going on in food technology departments on controlled atmospheres for the storage of crops. Many of the crops we are able to buy out of season, such as "apples in the springtime that are really pretty crispy and good," have been stored not just in cold but in highly controlled atmospheres in which the CO_2 level is higher and the oxygen is lower. Atmospheres are modified very carefully to not let those apples, bananas, or other fruit respire "because it's respiration that makes fruits go mushy and destroys them."

The ensuing discussion led again to economics. The most important point made in practical terms was the recognition of this form of 'cheap cold' since "usually the power costs of cooling and lighting in CEA are just too great; but, if you have free light and cheap cold"...more things become possible. Workshop Co-Chair Craven also reinforced participants' reflections on problems involved in planning a new project. "The problem with innovation is, if you're not getting an income at the very start of the innovative project, then everything you spend, particularly if you borrow money, can put you in a financial hole from which you may never recover....so, finding a product that you can sell immediately that is compatible with innovation...puts a high premium on crops which are produced in couple months time rather than products with a longer incubation time."

Workshop participants generally expressed their pleasure at the interdisciplinary approach taken at the Workshop, mixing plant physiologists with engineers and experts from other fields. As Pat Sullivan noted, "...creative thinking is essential for us to continue" in addressing models for future production projects. Clare Hachmuth added that listening to the Workshop discussion was 'extremely encouraging' and she expressed the hope that all the new ideas would eventually result in new projects and possibilities at NELHA, such as the use of seawater in the storage of agricultural products. Howard Takata shared his pleasure at seeing action being taken towards exploring diversified agriculture in an unexpected place.... Even the cameraman (video taping the session for Marlon Brando) noted that "I wish that every city had a 3,000 foot dropoff of ocean water..."

Tom Daniel led the discussion towards a different focus, regarding how to get all those elements closer together so needed progress in cold ag research can be made. While it was agreed that much research is yet to be done and NELHA as a facility "would be glad to provide research space," he expressed a concern that, first, perhaps the field setting at NELHA would not be appropriate for the kind of experimental areas that have been discussed, "because conditions could be replicated in the laboratory in terms of control," and, second, the kind of "research that needs to be done is too expensive and much too complex to be done by the people who are going to do the production," i.e., those starting innovative business ventures at NELHA. Third, the challenge yet remains to attract those academicians who would carry out the 'right' research to get interested enough to seek funding/grants to do so.

Dr. Daniel concluded that the furthering of research in this area will be a matter of encouraging the academic people to come up with a plan with which Co-Chair Weaver concurred, adding that "a lot of work gets done" even without much money by getting students involved for academic credit. Little by little, what is needed is effort to engage a few enthusiasts, starting with putting these ideas out into the public realm to get more people interested.

Marty Vitousek shared anecdotal information regarding the unpredictable nature of growing plants in test conditions. In the early work (mid-1980's) he did to test cold ag strawberry growth at NELH, he had contacted the Aaron Strawberry Farm in Ohio to get a couple of hardy varieties to try. It turned out that those varieties did worse than the Sequoia variety from California that he had started with.

Cary Mitchell sought to clarify technical aspects of cold ag proposed earlier by Craven by noting that he had made some very interesting thermodynamic speculations of a facilitated upward diffusion of inorganic minerals in response to a ΔT gradient. If surface pipes are condensing water and it is percolating downwards throughout the soil profile...the question (asked) before...about separating the two might be(come) very relevant....Could the mass flow of downward percolating water oppose this facilitated diffusion along the temperature gradient upward and blunt it somewhat. Would it be better to channel this condensate water so it is funneled downward and not through?

Craven replied positively, and further elaborated that when he and Jim Russell had sat down to decide how to grow cold ag strawberries, that was exactly the mechanism they talked about. They had contemplated a mechanism whereby the downward flow of water would collect the minerals and bring them to the root zone and the root zone, which would be cold and would operate as a wick with the warm above.

Mitchell pointed out that "the roots have got to be between; if you've got the cold pipe below, at some point the percolation and the facilitated diffusion are going to meet, and if that's below the root zone, it may retard."

Craven added that if you're looking for a physical mechanism that will take the minerals out of the soil and take them down below the root zone and then put them in the root zone in such a case as the thermodynamics will now bring them up through the plant, if that's an important mechanism, and as "we engineers would suspect it was," Mitchell would be 'absolutely right' in that "you would have to watch out that you don't end up with very, very simple changes in the flow pattern which (could) defeat the whole purpose" of the set-up.

Dr. Glenn noted that the root zone of the plant will adapt to the water situation it finds. Dr. Mitchell then elaborated on his concern that "in terms of the temperature gradient, if you're putting 5° C water through and the roots would rather have it a little higher than that, they will grow less into that zone and they will grow where it's more optimum. They will find the optimum temperature. You've got two things there, as you said, they'll go for the optimum moisture and oxygen content but also temperature so it becomes very complicated."

Dr. Glenn reemphasized Tom Daniel's concern for a look at the larger perspective, in this case the "enormous quantities of water to utilize" when dealing with the deep ocean water to be brought ashore and that using "cool ag" and "Cold Ag" in field crops might ultimately be the greater potential...all the way up to direct use of the seawater. Dr. Craven agreed and reemphasized that the large scale experimental production of *Salicornia* discussed previously used freshwater cooled with a heat exchanger to periodically flood the fields during certain phases of growth. The amazing hardiness of true halophytes such as *Salicornia*, cultivated for its oil yield, was also discussed. Dr. Glenn pointed out that there are thousands of species that can grow in undiluted seawater, in fact, certain *Salicornia* field crops were irrigated in the same selected fields for up to eight years using 40 ppt seawater from the upper Gulf of California.

Weaver and Chang put in a pitch for integrated systems which incorporate other water uses such as growing opihi (limpet) in an integrated system. Dr. Chang revamped the progress of the discussions during the Workshop, pointing out that when it started, "we were actually talking cold water agriculture," which was quickly shortened to "cold agriculture." Then "after that we just concentrated on the cold and forgot about the water altogether." He reminded the group that "the water minus the cold is still a useful resource and anybody who wants to design a system efficiently will have to somehow make use of the water itself as well in order to derive the maximum efficiency out of it."

Multiple uses of the same water brings into play a redefinition of how to use and price the resource. After one person uses the water it may be perfectly good for somebody else to use. Although current NELHA policy limits water use so it cannot be resold, this has nothing to do with physical efficiency of a system. To get maximum efficiency and maximum economic return it should be noted that most likely the system will have to be integrated and that we are talking about only a part of the system in this workshop. "We're not addressing the aquaculture part of the possible integrated coldwater agriculture/aquaculture system."

A question was raised to define "Cold Ag" so Dr. Craven stated that it is "agriculture that optimizes on the availability of low cost cold." Dr. Mitchell added that "it is is a theory which is testable by the scientific method." Craven further elaborated: "But in practice, if you have a "Cold Ag" farm, then that means you would be doing agriculture as opposed to aquaculture but you would be utilizing deep ocean water as a source of cold in which the cold is used as a resource for many many processes including the plant physiology in the agriculture....You can have cold ag because it turns out that it is economic to pump the coldwater up solely for the purposes of agriculture. It's probably not wise because there are so many other uses for it but we're discovering that there are some close-to-profitable or profitable activities in which the agriculture pays for the pumping of the water itself. This came up in the discussion of the amortization of the pipeline as to whether the agriculture people should pay 8 cents a thousand gallons or whether they should pay 32 cents a thousand gallons or 16 as a function of how many times the water is used as to what each user should legitimately pay for amortization of the capital equipment.

How to set up a pricing system for multiple use of the coldwater by different tenants turns out to be quite a dilemma. One cannot simply charge per BTU as in other energy systems since there are numerous characteristics of the water and "as the coldwater goes through each application, it comes out with changed characteristics. If it comes out cold but impure, so what, you sell it to someone who wants it for cold. If it comes out warm but pure, you sell it to someone who wants it for pure and so on and so forth. And so therefore, through each pass, you have a whole brand new resource in terms of these resource characteristics we are talking about which is purity, temperature, nutrient content, salinity, mineral content, conductivity."

Co-Chair Weaver reemphasized the exciting potential of cold ag by citing the opinion of a friend, Andrew Benson, well-known in biology "because he worked out the carbon path in photosynthesis." On learning about cold ag he insisted, "Forget about power, put all your energy into cold ag!"

The potential to use deep seawater to cool freshwater where there is plenty was also brought up. Systems that could afford to do this would have increased flexibility in the use of the cold as they could minimize the corrosion potential of seawater by using the freshwater in heat exchangers.

A request for a simple explanation of cold ag applications in lay terms was made. Craven offered that "the condensate on the outside is very similar to trickle irrigation, so you can tell them it's an alternative to trickle irrigation but much less expensive."

Another look at the concept of heat transfer brought an interesting discussion which is quoted verbatim here:

Mitchell: "When Andy Benson was saying forget about the power and go for the cold, I was just about to say something that was just the opposite. We've been emphasizing the effect of cold temperature, i.e., temperature on the growth of plants at this workshop then today we have more or less formalized the introduction of CEA, the control of temperature is part of that. There are degrees of CEA. I consider greenhouses to be partial CEA. You put artificial lighting into greenhouses and you take it to another level. You also create a further heat rejection problem. I think another thing that should be addressed and brought full cycle on this is artificial plant growth lighting in opaque structures. They might be towers and use part of this cold water to generate power and use a little bit of that power to drive the proper plant growth lamps. Then you don't have seasonal problems, you don't have daily problems with cloudiness, you can optimize photoperiods for productivity and you can truly optimize a crop with respect to the five cardinal factors of plant growth which are light, temperature, water, nutrients, and atmosphere. But you need containment for this and you need artificial lighting, and we haven't really brought all that together yet. The power comes in."

Craven: As I've said before, you have available right now one and only one power plant which is the General Electric 1 MW OTEC plant and even that is not available for 12 months or so. When you have that, then you have power for pumping and a little surplus for lighting or things of this kind. And in the lobster farm's proposal the net surplus power is coming out as zero; it will be used entirely in the process. But the point to make for the workshop is that the power system is a bonus that you don't really have to have because most of your power saving comes in substitition of cooling or refrigeration. And that substitution is such a large large component; we haven't covered that section of this report yet to talk about the use of deep ocean cold in agricultural processing and ag production; we're still in the plant physiology part of this report.

Mitchell: I told you that you could increase the annual production of strawberries 12- to 16-fold by going to complete CEA with artificial lighting including photoperiod, quality, and all of that. You would create two new heat rejection problems; two power requirements, one to drive lamps and one to reject the heat generated by the lamps.

Craven: It's worth it if I use the coldwater for the heat rejection part; it's not worth it if I use the coldwater for generating power for the production of the heat....it isn't very expensive to produce hot; it's very expensive to produce cool. There is an asymmetry between the cost of cool and the cost of hot. Hot is cheap and very hot is cheap; all you need is some oxygen and some fuel and you burn it and you get hot. But cold is very very expensive and operates on the Carnot efficiency cycle; it's the inverse of OTEC. But I think you put your finger on a concept that the world doesn't understand metaphorically and that is that the metaphor for energy in our world is hot but no energy is produced by hot. All energy is produced by ΔT. And the thing that the world is deficient in is cool.

For example, there was a proposal to put in an electrical plant in the coal fields of the Rocky Mountains, called the Mine Mouth Electrical Generation Plant. And they made all the plans for the electricity generating plant...and discovered that they couldn't put it in because there wasn't enough water in the rivers to come down and cool the plant. And the fact that you can generate hot means nothing whatsoever; you've got to condense on the other side of the condenser. So you can see one or two major power systems that have been denied because of the absence of cold. So if we can somehow put that into this energy picture that the world efficiency in energy production is cold, not hot.

Chang: I think this (statement) should go into the purpose (of the workshop). I think that you instinctively just add the cost of the cold since the cost of producing cold is many many times the cost of hot....The reason we are talking about cold agriculture is the expenses of producing cold. And here we have an inexpensive cold. It's not that the cold is inexpensive; it is because of the expenses of producing cold.

Craven: That is exactly right; because the alternative to using cold is compressors and astronomical cost.

Craven: When I was talking to Marlon Brando about this, Marlon said what do I care about industrial cooling if all I want to do in my island is produce fish. I said the most expensive thing you will have if you produce fish is putting them in the chiller so they don't spoil. And I said if you go and look at costs of coastal fishing anywhere, the big cost is in the preservation and chilling of the fish. And that's a gigantic cost; it's not a little cost. And so once you eliminate that cost, then all of a sudden you have a process of fishing that has two major expenses, and one is the fuel for the fishing boat which could be eliminated by coastal fishing and the other, which is the major expense is the chilling.

The discussion then turned to summarize what was learned from Dr. Chang's presentation on mushrooms. It was agreed that mushrooms are one of most valuable resources in terms of a) converting waste (includes bagasse, wood chips, coffee husks, koa wood chips) into useful products, including soil conditioner and compost, and b) the best mushrooms require a significant ΔT for quality and c) there are some mushrooms that have an absolute cold requirement for fruiting. Therefore, mushroom production seems to be ideal for Hawaiian environment and for tropical environments wherever tropical environments have accessibility to deep ocean cold. The potential market for mushroom consumption in Hawaii and elsewhere is also great. According to Dr. Chang, the average American eats about 1.5 kilo per year while the Chinese eat 3 kilos/yr. A statistic is not yet available for Hawaii, but would be relatively easy to calculate. The price in the US market is about 3-6 dollars wholesale, so 12-15 dollars per pound retail. Mushrooms can be used for medicine and for food, are 'low cal' so conceivably could be marketed as 'diet mushrooms' in some nouvelle cuisine. More varieties seem to be on the market these days as compared to 4-5 yars ago, according to Dr. Weaver.

Significantly, a cold pretreatment prior to shipping can extend the shelf life and quality of mushrooms. As a product, they are perishable; i.e., limited in range of shipment. In Hong Kong, mushrooms are routinely put into a cold room for four hours to increase the shelf life by several days while improving the quality. They are not shipped cold. The cold pretreatment will minimize oxidation and reduce respiration so therefore the product will last for a week in the supermarket. If they are not chilled, the mushrooms will open during shipment and be of lower quality. Dr. Chang and colleagues have been working on this process for a few years. A modified CEA ship storage is also used. In shipping mushrooms in Korea and China they are packed in dry ice for two reasons: to lower the temperature and to increase carbon dioxide to reduce respiration.

The species most suitable for starting here, according to Dr. Chang, are shiitake and *Flammelina*, if really low temperatures can be obtained. Michael Harburg pointed out that there are also local types that are delicacies. The black tree fungus is popular with Chinese and Filipinos. *Flammelina* travels well, however, there are already people growing them on a large scale, so his company will focus on the local market. Dr. Weaver projected that "the market is going to be good enough to ship anywhere" and that they should be relatively inexpensive to produce. At Keahole, there is also the advantage of being close to the airport for international shipments. Products can get to Japan in 8-10 hours. The potential for the mushroom market is large as is the island's capacity to produce them.

Dr. Craven summarized the use of cold in processing. First, he pointed out, it is impossible to think of any agricultural processing system that doesn't have extensive use of cold in almost every aspect of packaging, processing, and transport of product. Cold is also needed in fermentation, has been shown to be cost effective in use for distillation of alcohol from sugar cane using no outside sources of energy. Flower industry could also use cold to good advantage. Cold was used at NELH for CO_2 scrubbing process, as well as in air conditioning all of the buildings the Workshop participants toured. Complementary savings associated with the aquaculture was also noted. The fact that aquaculture can use same water used for agriculture is another important point. It was also noted that possible contamination of multiple uses of the deep seawater may not be a concern if the water continues to be so cheap (at 8 cents per thousand gallons).

A final topic of interest was the technology of LEDs (light-emitting diode) for use in closed ecological life support systems, brought up by Drs. Weaver and Mitchell. LEDs are being used as a source of light to support photosynthesis. The entire output is usable by the plant for photosynthesis with very little heat. They are very economical to run compared with fluorescents and incandescents to get white light and come in several colors. Incidentally, although they can use any light, red light is what plants like best.

LEDs are commonly used in alarm clock radios and other household items. They're very cool, solid state, and their diodes are made from gallium arsenide. Any monochromatic wavelength can be created in an LED. For example, you could make one that has emission rated at 680 nm and nothing else with less than a 20 nm band width. So, one could design LEDs with maximum absorbance of a particular plant pigment you are interested in. Some arrays have been created in which the amperage to the chip is modulated to even get white light.

Right now red ones are very cheap and blue ones very expensive; however, this technology is moving very rapidly and within a year, it is projected that blues are going to be economical too. They can be wrapped around the plant or even cut into sheets. If you can design the LED at the maximum absorbance of the pigment targeted, you wouldn't have to have high intensities to saturate the mechanism and therefore don't need as much power. They have lifetimes in excess of 100,000 hours without decreasing output. There is some heat produced at the node but not much connected with the element itself. It is a promising technology which, while not at commercial scale yet, is likely to become widely available in just the next few years and is the up and coming thing in plant growth lighting.

Comment: You can be the heroes of tomorrow if you change the way we create energy.

POST-WORKSHOP COMMENTARY: CEA AND "COLD AG"

By Dr. Cary A. Mitchell
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Controlled-Environmental Agriculture (CEA) is a fledgling growth industry with great potential for efficient production of safe, high-quality food crops. Presently, the technology is cost-limited by power for plant-growth lighting and by labor. CEA capitalizes on absolute control of the five Cardinal Factors of Plant Growth, i.e., light, temperature, water, nutrients, and atmosphere. "COLD AG" participants are coming in the back door of CEA by discovering one aspect of one Cardinal Factor and its great potential for production of temperate-zone food crops outdoors in the tropics.

Indeed, the concept of rootzone cooling permits adaptation of certain species (e.g., strawberry) to successfully withstand supraoptimal shoot temperatures and the produce an acceptable, high-quality yield. However, the potential for unlimited quantities of deep cold seawater (i.e., 5–7°C) to help provide the ΔT for electrical power generation, without upsetting the ecological balance of the oceans (because cold seawater is a naturally renewable resource) may in fact create fantastic potential for complete as well as partial CEA in tropical areas with a steep coastal shelf.

Electrical power derived from ΔT could be used to drive plant-growth lamps, and cold seawater could be used directly for heat rejection in illuminated plant-growth compartments where the "greenhouse effect" needs to be accounted for and where temperature needs to be controlled for optimum growth. In other words, CEA chambers (towers, warehouses, or whatever) may not need to be limited solely to control of rootzone temperature. At about 19°N latitude, photoperiod on the Big Island of Hawaii fluctuates between about 11 and 13 hours of light per day throughout the year. Thus, to productively grow long-day crops with a critical photoperiod longer than this range, or to grow day-neutral crops that respond to the total integral of daily photosynthetic energy, it would be necessary to use supplemental lighting at the least, or all artificial lighting at most to produce high-quality, marketable products.

By combining the art and science of hydroponic culture (provides the Cardinal Factors, water and mineral nutrients) with chamber containment of crops (provides temperature, humidity, and CO_2 control), it is possible to produce crops much faster than in the field and usually with greater yield per cycle than in the field. With additional control of photoperiod and light level, many more cropping cycles per year can be achieved by CEA, which is independent of prevailing weather conditions in the field.

Many consumers appreciate hydroponic CEA products (e.g., lettuce, spinach, herbs) because they are dirt- and sand-free, and, because of appropriate sanitation and exclusion practices, usually also are insect and pest-free. Hydroponic culture of short-statured crops such as strawberry has been the subject of much horticultural research in recent years, and its use as a production practice for strawberry appears feasible. Recirculating hydroponic culture is one aspect of CEA that would be very amenable to separate control of root and shoot temperatures, as well as the controlled delivery of nutrients and chemicals to roots.

If partial CEA in the form of root cooling is found to be sufficient to make tropical cultivation in the open field competitive with products shipped in from the mainland or from other areas in a global market, that is fine, but if local demand and export demand increases rapidly due to the competitive economic edge of ΔT agriculture (e.g., production of high-value floral crops), then coastal acreage might become limiting in the future. In this case, development of high-rise, or vertical underground, structures enclosing total CEA operations powered by ΔT might be pursued, depending on what the State of Hawaii wants the coastline to look like.

FIELD NOTES FROM THE COMMON HERITAGE CORP. "COLD AG" COOPERATIVE GARDEN

What began as the Common Heritage Corp. (CHC) Demonstration Garden in its first phase (c. 1991-2) evolved naturally into the CHC "COLD AG" Cooperative Garden in its second phase. Since 1992 and the "COLD AG" Workshop, a cooperative approach to gardening yielded a variety of outcomes and discoveries under the hot Kona sunshine, nurtured by the cold soils and condensation produced by cold deep seawater pipes buried under the soil.

The following notes document some of the activities associated with the CHC "COLD AG" Cooperative Garden. A partial list of the many varieties of vegetable and flowering plants tested in the garden is also included in this section.

The following text was distributed in flyers by the Common Heritage Corp. to arouse community interest and solicit volunteer gardeners for a cooperative garden using cold deep ocean water and "COLD AG' technology.

ANNOUNCING:
A "COLD AG" COOPERATIVE GARDEN

Common Heritage Corp. has demonstrated the use of deep ocean coldwater as a means of creating microclimates through the local application of low cost cold. It has also been demonstrated that the uniqueness of the microclimates in the tropical desert provides a basis for organic gardening of a wide variety of plants which are normally grown in temperate or alpine regions of the world. Strawberries, asparagus, lettuce, and alpine flowers have already been produced by earlier investigators. It has also been determined that impoverished third world coastal desert communities are in immediate need of family and village size gardens which require minimum attention and are preferably organic.

For purposes of development and demonstration Common Heritage Corp. will sponsor a "COLD-AG Cooperative Garden" on its premises at the Natural Energy Laboratory of Hawaii. Each member of the cooperative will be assigned a plot in the garden for the purpose of growing a crop or crops of their choice. Members will work with Dr. John Craven, President of Common Heritage Corp. to determine the distribution of pipes, fans, and heat exchangers for the establishment of optimum seasons, photoperiods and microclimates for the chosen crops. All costs except for labor and transportation will be borne by CHC. Produce will be the property of the cooperative to be distributed as determined by the cooperative. Produce may not be sold and is for the personal use and consumption by members of the Cooperative.

Volunteers to participate in this Cooperative are solicited. If interested please call Oahu 377-1530 or 373-9369 and leave your name and phone number. An organizational meeting will be held as soon as a sufficient number of interests have been expressed.

Tell me tell me if you would
How many herring grow in the wood?
I'll tell you if you'll tell me
How many strawberries grow in the sea.

THE "COLD AG" EXPERIENCE

Crop Varieties Tested by Common Heritage Corp. at the "COLD AG" Cooperative Garden, NELHA, Ke-ahole, Hawaii September 1993 through May 1995

John P. Craven, President *Kevin Sue Rohan, Principal Agriculturist*

VARIETIES TESTED	PLANTING METHOD	OBSERVATIONS
VEGETABLES		
Carrots	seed	sweet-quality
Celery	seed	quality
Radish	seed	quality
Corn	seed	poor results
Beets (golden)	seed	sweet-quality
Beets (red)		
Hawaiian Sweet Potato	spud	not enough data
String Beans (Blue Lake)	seed	poor results
Sweet Peas	seed	poor results
Cucumber (lemon chinese)	seed	quality-rapid growth
Green Peppers	seedling	quality
Jalapeno Peppers	seedling	quality
Banana Pepper	seedling	quality
Anaheim Peppers		
Sweet Red Peppers	pests	
Cayenne		
Paprika		
Leek	seed	good results
Sweet Bunching Onion	seed	good results
Yellow Onion Bulb	bulb	good results
Ebenezer		
Garlic Chives		
Common Chives		
Garlic	clove	not enough data
Spanish Onion	seedling	good results
Broccoli (green sprouting)	seedling	good results
Cauliflower		
Cabbage (Savoy)	seedling	large heads-sweet
Chinese Celery Cabbage		
Brussels Sprouts	seedling	poor results
Strawberries	existing plants	good results-sweet
Okra (Clemson)	seed	quality
Okra (Spineless)		
Mustard Greens	seed	good results
Bok Choi	seedling	quality
Tai sai		
Artichoke	seedling & root	good results
Rhubarb	root	poor results
Radichio	seedling	good results
Rockett	seed	not enough data
Asparagus	year old root	promising

VARIETIES TESTED	PLANTING METHOD	OBSERVATIONS
VEGETABLES (Continued)		
Acorn Squash	seed	poor results
Zucchini	seed	good results
Pumpkin Hawaiian	seed	quality
Molokai Watermelon		
Hawaiian Gourd (Ipu)	seed	quality fast growth
Eggplant	seed	not enough data
Tomatoes		
Cherry	seedling	quality sweet
Roma	seedling	quality sweet
Yellow Pear	seedling	quality sweet
Beefsteak	seedling	quality sweet
Luffa Sponge	seedling & seed	good results
Spinach	seed	not enough data
Celeric Dolvie	seed	moderate
Swiss chard (red)	seedling	good results
Giant Red Mustard	seedling	poor results
Green Chard		
Lettuce		
Oak leaf	seedling	good results
Buttercrunch	seedling	good results
Bib	seedling	good results
Loose leaf	seedling	good results
Mizzuno	seedling	good results
Ruby Red		
Black seeded simpson		
Red Sails		
Endive, Green Curled	seedling	good results
HERBS		
Florence Fennel	seedling	good results
Spilanthies	seedling	quality slow growth
Chamomile	seedling	quality slow growth
Shiso	seedling	poor results
Lavender	seedling	poor results
Peppermint	seedling	good results
Spearmint	seedling	good results
Coriander (cilantro)	seed	quality fast growth
Nasturtium	seedling	poor results
Marigold	seed	good results
Chrysanthemum (feverfew)	seedling	quality slow growth
Black lovage	seed	quality slow growth
Borage	seedling	good results
Dill	seed	good results
Catnip	seedling	poor results
Catmint		
Sweet Marjoram	seedling	poor results
Basil (5 varieties)		
FLOWERS		
Alstroemeria	seedling	good results

VARIETIES TESTED	PLANTING METHOD	OBSERVATIONS
Azalea	2 qt size transplant	poor results
Carnation	seedling	good results
Calla Lilies	bulb	good results
Gladioli	bulb	good results
Cosmos	seedling	poor results
Allysum	seedling	poor results
Celcsoia	seedling	good results
Hawaiian Cotton		
FRUIT TREES		
Apple	transplant	no data yet available
Nectarine Dwarf (2)	transplant	no data yet available
Pear	transplant	no data yet available
Peach	transplant	no data yet available

May 11, 1995, 0900

PRESS RELEASE

The BLUE-GREEN Revolution

KAILUA-KONA, HAWAII. In the early 1950's, the Indian government in collaboration with the United States, initiated the "Green Revolution." The Rockefeller Foundation, the Ford Foundation, and the American universities spearheaded the effort establishing many land-grant type universities in India. Because of the revolution, India has become a major exporter of cereal crops. Attempts to start a "Blue Revolution" began in the 1970's. To date it is far behind, although the many projects at NELH indicate that it too will have a significant impact on humanity.

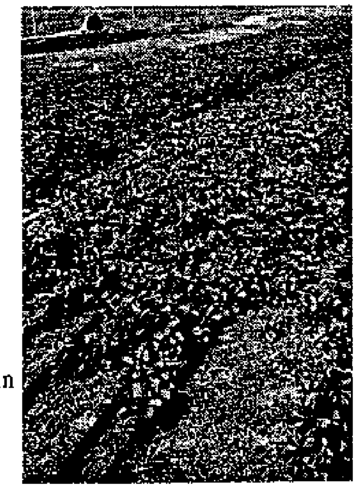
On January 22, 1994, the Honorable Senator Richard Matsuura was a speaker at the dedication of the Common Heritage Corp. coldwater agriculture garden. Senator Matsuura, a Ph.D. in horticulture and a major contributor to the Green Revolution challenged the CHC to break dormancy with temperate zone crops raised on the Kona desert. Dr. John Craven and Ms. Kevin Sue Rohan of the corporation accepted the challenge and encouraged Mr. Shane Rohan of the Cyanotech Corporation to plant five dwarf fruit trees. One of these, a pear tree, has been induced to flower and fruit four times in a single year. The first fruits have been harvested and have been found to be very sweet. The second fruitings have ripened and samples are available for taste and one of the remaining pears may indeed be on the tree at the time of this release. The third and fourth fruiting are now maturing. The flowering have been induced by the application of cold (10 degrees C) from deep ocean water which flows in coils about the roots of the tree. The interruption of this cold has caused the trees to shed leaves and to appear to be dormant. The restoration of this cold appears to break dormancy and induce flowering and fruiting. If this is indeed true, then a major breakthrough will have been achieved in tropical agriculture and in particular for agriculture on previously barren coastal deserts. Senator Matsuura suggests that this could usher in a "BLUE-GREEN" revolution of coastal 'gardens of Eden' environmentally sustained by the globally renewable cold of the deep sea.

At the urging of Senator Matsuura, representatives of Common Heritage Corp. and the University of Hawaii have indicated that they will initiate a series of controlled experiments to determine if the breaking of dormancy has in fact taken place and has been the result of the application of cold.

Such a "BLUE-GREEN" revolution will of course include the many other products under development here at NELH. Senator Matsuura was the floor leader for the revitalization and reorganization of the Natural Energy Laboratory in 1980. Dr. Jack Davidson, Director of Sea Grant, has been the chief University sponsor of research conducted at the Laboratory. Mr. Gerry Cysewski of Cyanotech has been one of the first corporations to demonstrate that with the use of deep ocean water, a green crop of *Spirulina* can be produced on an environmentally sustainable and profitably sustainable basis. Other corporations such as Kona Cold Lobster have demonstrated that crops of the Blue Revolution can be similarly produced and sustained.

1. Greenhouse-grown hydroponic lettuce set in sheets of styrofoam and floated in nutrient-filled raceways for a research project in Tucson, Arizona.
Photo contributed by E. Glenn.

2. Close-up of individual lettuce plants taken from styrofoam sheeting material in the Arizona hydroponics project.
Photo contributed by E. Glenn.



3. "COLD AG" strawberry beds in the first phase of the CHC Demonstration Garden at NELHA in Keahole, Hawaii. The temporarily untended plants are growing along the condensate-laden cold deep seawater pipes on the bed surface. Cold seawater pipes were also buried underground in these beds.
Photo by J. Craven.

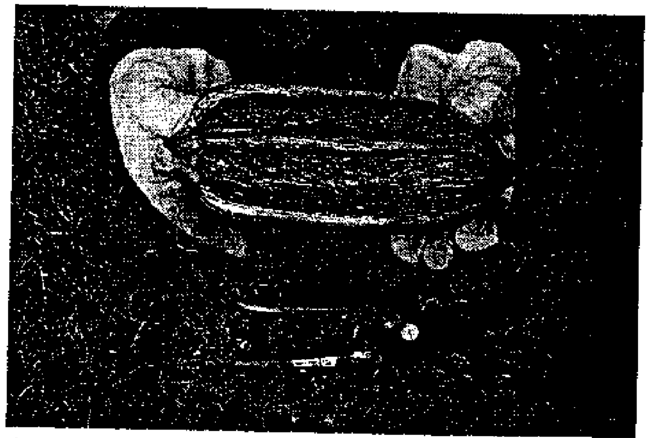
4. A sweet "COLD AG" strawberry harvested from the first phase of the CHC Demonstration Garden. *Photo by J. Craven.*

5. "Cold ag" bed in the second phase of the CHC Demonstration Garden, now the "COLD AG" Cooperative Garden, showing exposed deep seawater pipelines, all of which will be buried in the soil.7777 .
Photo by B. Lee.

6. "COLD AG" strawberry bed in the CHC Cooperative Garden showing subsurface pipe. All beds utilized buried cold seawater pipelines; no exposed surface pipes were used in this phase of the Garden. *Photo by B. Lee.*

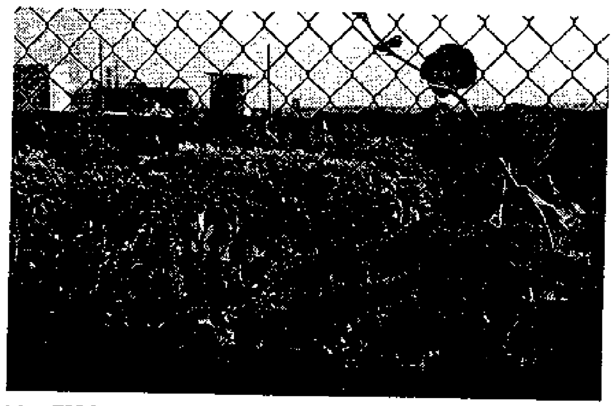
7. Thriving vegetable bed in the CHC "COLD AG" Cooperative Garden
Photo by J. Craven.

8. Mulched vegetable bed in the CHC "COLD AG" Cooperative Garden. *Photo by J.A. Craven.*



9. Squash harvested from the CHC "COLD AG" Cooperative Garden. *Photo by J.A. Craven.*

10. Endives in the CHC "COLD AG" Cooperative Garden. *Photo by J.A. Craven.*



11. CHC "COLD AG" Cooperative Garden vegetable beds thriving in the Keahole coastal desert with the ocean horizon visible in the background. *Photo by B. Lee.*

12. Vegetable and carrot bed from CHC "COLD AG" Cooperative Garden. Insulation by addition of mulching resulted an additional drop in soil temperatures. *Photo by B. Lee.*

13. CHC President John Craven and volunteer cooperative gardener Gary Owens with fresh lettuce harvest from the CHC "COLD AG" Cooperartive Garden. *Photo by B. Lee.*