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Genetic engineering may increase effectiveness of the Japanese beetle pathogen, *Bacillus popilliae*

By Douglas Dingman

Recombinant DNA (rDNA) technology has arisen from research in the fields of molecular biology, microbiology, biochemistry, immunology, virology, and cell biology. Molecular biology, the primary discipline for this technology, seeks an understanding of cellular processes in order to develop new tools for cellular research. From the use of these tools, new frontiers in biological research are opening.

New frontiers are currently appearing in medicine, criminology, agriculture, and pharmacology, to mention a few. In medicine alone, researchers are developing faster and more sensitive methods to detect and cure disease. These new frontiers are possible because the methods developed allow monitoring of biological processes at the level of cellular information and control — the DNA (Deoxyribonucleic Acid) molecule.

DNA is a helical molecule which is the genetic material of all cells (Figure 1). This molecule directs every biological process in a living cell. Genes, informational segments of the DNA, direct these processes through a complex process called *transcription*, during which they are copied into RNA (Ribonucleic Acid) molecules. An RNA molecule can then be used to produce a protein through another complex process called *translation*. Proteins comprise the structural and functional framework of a cell.

The promise of rDNA technology is realized through the ability to engineer DNA (Figure 2). By cutting (*restricting*) a very large DNA molecule into short strands and splicing (*ligating*) one or more of these pieces of DNA (containing information for a particular cellular process) into a different DNA molecule, a new DNA molecule can be created. This new molecule, now containing the inserted splice, can be placed into a cell. That cell will now have the information needed to express the physical characteristics specified by the splice of DNA. Thus, rDNA technology allows the cellular information contained within DNA to be manipulated. DNA

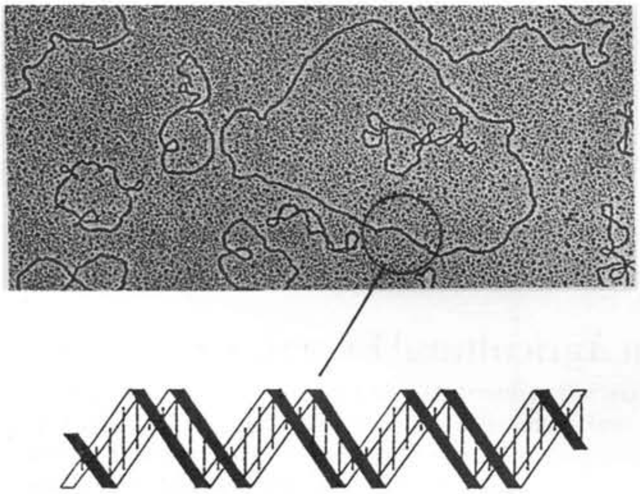

Figure 1. An electronmicrograph of DNA molecules. The schematic representation shows the double-helical nature of an enlarged segment of DNA.

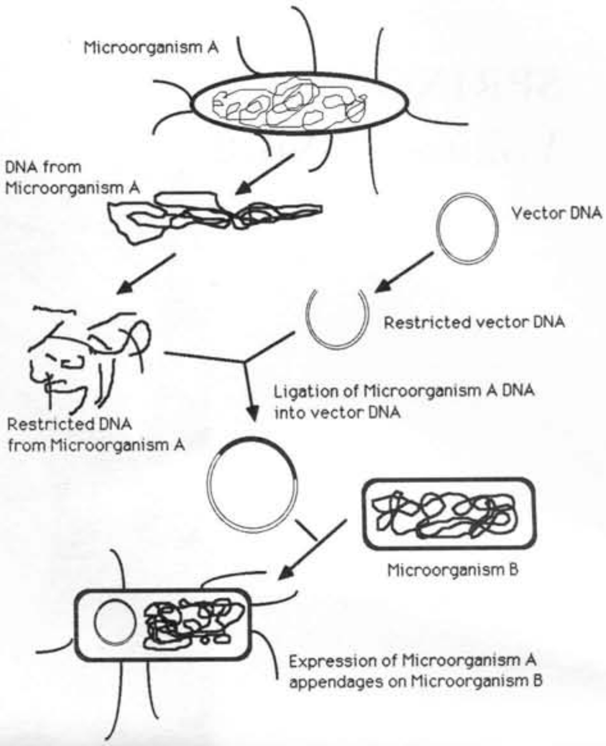

Figure 2. Recombinant DNA methodology for genetic engineering.

can be taken from one organism and placed into another or it can be modified and placed back into the original organism.

At The Connecticut Agricultural Experiment Station, rDNA technology is being used to study the pathogenic nature of *Bacillus popilliae* (Figure 3), a sporeforming bacterium which is the natural cause of milky disease in Japanese beetle (*Popillia japonica* Newman) larvae. Improved use of this microorganism as a biological control agent for the Japanese beetle may be gained from this research.

An infection is initiated after a larva ingests soil which contains *B. popilliae* spores. Spores are the dormant form of certain types of bacteria. Once in the intestinal tract of the insect, the spores germinate to produce bacterial cells. These bacteria bind to the *epithelial* cells lining the inside of the intestine and actively penetrate the intestinal wall. Within 1-2 days following ingestion, *B. popilliae* traverses the intestine and invades the blood cavity, causing an infection to develop which kills the larva. Prior to the death of the insect, spores are produced which are released into the soil as the larva decomposes.

Although a commercial insecticide made of *B. popilliae* spores is available, use of this bacterium as a bio-insecticide has several problems. *B. popilliae* is difficult to grow in the laboratory, and the microorganism dies quickly unless carefully cultured. Also, the microbe is unable to produce spores when grown outside of the insect. Currently, spores are produced commercially by manually infecting *B. popilliae* into live Japanese beetle larvae, a costly, labor intensive, and inefficient process. Another potential drawback is the apparent diminishing ability of *B. popilliae* to cause milky disease in Connecticut.

The key to overcoming these problems and using this bacterium more effectively resides in using rDNA techniques to decipher the process of adhesion to and penetration of the intestinal wall, and then using this knowledge to improve the pathogen's effectiveness.

DNA from several strains of *B. popilliae* have been isolated and cut with a *restriction enzyme* (a biological tool used to cut DNA). The resulting numerous small fragments of *B. popilliae* DNA were individually inserted into DNA molecules of a *bacteriophage* (a virus which infects bacteria). This allows for easier manipulation of *B. popilliae* DNA and provides the capability to isolate a gene of interest from the many other *B. popilliae* genes. Work is presently being done to identify, from a large bacteriophage population, the single virus which contains *B. popilliae* DNA having information for adhesion to and penetration of intestinal epithelial cells. Once this bacteriophage is identified, the *B. popilliae* DNA will be isolated and characterized to determine the proteins it encodes. These proteins will be studied to see how they are involved in *B. popilliae* pathogenicity. This will require a determination of how they interact with insect intestinal epithelial cell proteins and where they reside in the bacterial cell.

If the isolated *B. popilliae* DNA encodes factors for pathogenicity, transfer of this DNA to another bacterium (one which grows and sporulates better than *B. popilliae*) may result in this other bacterium becoming pathogenic to the Japanese beetle. My preliminary datum indicates that *B. popilliae* DNA is expressed (transcribed and translated) in *Bacillus subtilis. B. subtilis* is a sporeforming bacterium which is easier to grow than *B. popilliae* and sporulates well on simple nutrients. Much is known about the molecular biology of *B. subtilis*, making it an ideal organism for construction of a better insecticide. Should *B. subtilis* be made infectious to the Japanese beetle, the ease of growing this organism and production of its spores (which are more stable than those of *B. popilliae*) will result in a cheaper, more efficient insecticide.

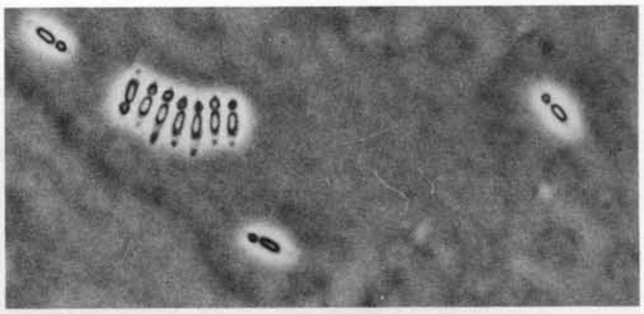

Figure 3. Spores (small ovals) and parasporal crystals of *B. popilliae* contained within bacterial sporangia. Photomicrograph courtesy of J. Hanula.

Use of this new insecticide will be safe. *B. popilliae* is known only to infect the Japanese beetle and a few other related beetles. DNA obtained from *B. popilliae* can only provide *B. subtilis* with the information to be infectious to the same insects as *B. popilliae*. Also, an infectious strain of *B. subtilis* can be manipulated so it will not grow in the environment.

In the future, rDNA technology may also provide some clues as to why *B. popilliae* is losing its effectiveness on the Japanese beetle in Connecticut. In addition, DNA from *B. popilliae* might be engineered to increase the strength of pathogenicity for the Japanese beetle or to have pathogenic effects on other harmful insects.

Protected plants produce red and yellow peppers prolifically

By Martin P.N. Gent

The ripe red or yellow sweet pepper commands a high price in retail stores. If such peppers would ripen early and if harvest could continue for 2 or 3 months, they would be an attractive crop for Connecticut farmers. However, two drawbacks limit the availability of ripe peppers to a short period in September and October. First, transplants are not set in the field until late in the spring, both to prevent frost damage and because the seedlings require warm temperatures to grow well. Second, the most popular cultivars, such as Calwonder and Yolo Wonder that were bred for California conditions, ripen late.

Row covers have been touted as a means for early production of many crops, including peppers. In the past, row covers of solid polyethylene sheet had to be supported above the plants by hoops and ventilated during the day to prevent high temperature injury. More recent introductions are the light and porous spun-bonded films, which can be draped directly over the plants and kept in place even on hot sunny days. Temperature under row cover averages 5 F above that in the open field. The extra growing degree days could accelerate plant development up to 2 weeks in the spring.

In 1987 and in 1988, I tested 8 and 16 sweet pepper cultivars, respectively, that were grown in the open and under spun-bonded polypropylene row cover. Peppers were transplanted at Lockwood farm in Hamden, CT on two dates. For the early planting, peppers were germinated in a heated greenhouse in mid-February and placed in an unheated cold frame to harden off in early April. On April 21 they were transplanted into the field into soil covered with black plastic mulch. The day after transplant half the peppers were covered with a film of spun-bonded polypropylene. This row cover remained continuously over the plants until it was removed on June 11, 1987 or June 24, 1988. Peppers were germinated in late March and placed in a cold frame in early May for the late planting on May 23. Row cover was applied the day after transplant and removed on June 29, 1987 or July 13, 1988.

Floating row cover accelerated vegetative growth and all cultivars flowered earlier. Row cover also accelerated fruit ripening of many of the early-planted cultivars. Those cultivars that benefited most from row cover started producing on July 25 and kept producing until the end of the season. The same cultivars grown in the open started producing later, on August 9, and the majority of the fruit ripened in a shorter interval. For example, the accumulated yield and the yield per pick for the cultivar Canape grown under row cover and in the open, are shown in Figure 1. Thus, row cover applied to an early planting resulted in both earlier production and a more constant production until frost.

Marketable yields varied widely among cultivars. The highest yielding were Canape and Gypsy, which produced about 1000 bushels/acre of marketable fruit in both 1987 and 1988. Golden Bell, Lady Belle, Parks Early, Stokes Early, and Super Set all yielded more than 800 bushels/acre in 1988. The cultivars least productive in 1988 were the California types, Golden Calwonder and Yolo Wonder, which yielded about 600 bushels/acre.

Table 1 illustrates the yield of marketable ripe or colored peppers picked from the various cultivars by mid-August, a date earlier than peppers would normally ripen when grown under conventional cultural practice. There were significant differences between cultivars in the early yield of ripe peppers in response to row cover. Canape, a cultivar with blocky green peppers turning to a uniform red at maturity, ripened 8 and 12 days earlier in 1987 and 1988 when planted early under row cover, compared to plants grown in the open. Parks Early and Super Set were similar types that also yielded 10 days earlier. Gypsy, a cultivar with yellow elongated peppers turning red at maturity, ripened 6 and 12 days earlier in 1987 and 1988 when planted early and grown under row cover compared to plants grown in the open. Golden Bell, a cultivar with blocky green peppers turning golden yellow at maturity, produced ripe peppers early in both 1987 and 1988. This cultivar was an exception to the general rule that large-fruited cultivars tended to yield later and to respond less to row cover. The yields reported in Table 1 are considerably higher than the average of 460 bushels/acre for Connecticut. In part, the high yields resulted from intensive cultivation, and repeated picking at short intervals, which reduced fruit loss after ripening.

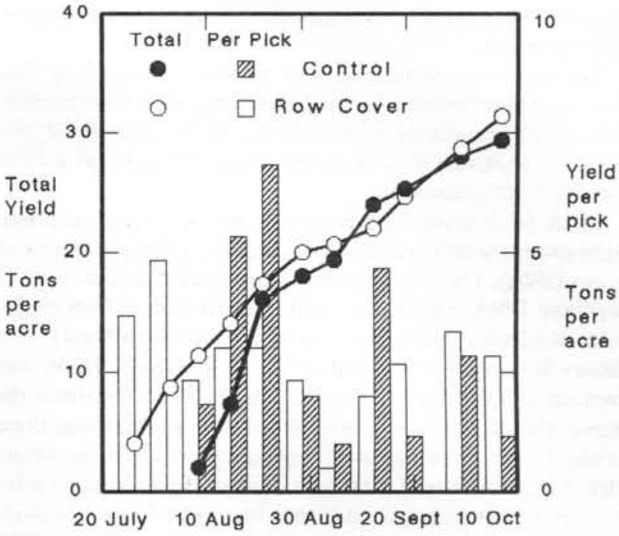

Figure 1. Accumulated yield (circles) and yield per pick (bars) of ripe Canape peppers in 1988 after growth for two months under row cover (empty) or in the open (shaded).

Row cover would benefit Connecticut farmers if the costs associated with its use were less than the extra value of production. For red or yellow peppers, the retail price remained at about $2.00/pound throughout much of the 1988 season and dropped to $0.50 to $1.00/pound only in September when local production was at its peak. Thus the economic benefit of row cover derived from production of ripe peppers in August when the retail price was high. I multiplied the difference in retail price between August and September, $1.00/pound, times the difference in yield by mid-August between plants grown under row over and in the open, 5000 pounds/acre for the most productive cultivars, to calculate the economic benefit of row cover. This calculation suggests row cover increased the retail value of ripe peppers by $5000/acre, which compares favorably with the extra production costs of $700/acre for the material, $500/acre for the labor to cover and

Table 1. Early and total yields of high quality ripe peppers from an early planting grown under row cover in 1987 and 1988.

					1987		1988	
Cultivar	Color Immature	Ripe	Maturity	Weight /fruit	Early Aug 14	Total Oct 8	Early Aug 15	Total Oct 12
				Ounce	Bushel/Acre		Bushel/ Acre	
Bell Captain	Green	Red	Late	5.8	22	346	-	-
Canape	Green	Red	Early	3.1	506	993	464	1095
Earliest Red	Green	Red	Early	2.5		-	73	614
Early Calwonder	Green	Red	Late	4.2	22	529	-	-
Golden Bell	Green	Yellow	Early	4.7	289	798	490	823
Golden Calwonder	Green	Yellow	Late	4.0	53	760	281	559
Golden Summer	Lt. Grn	Yellow	Late	5.4	-	-	195	747
Goldie	Yellow	Red	Early	4.6	-	-	177	596
Gypsy	Yellow	Red	Early	3.5	653	1008	504	1047
Lady Belle	Green	Red	Mid	5.5	-	-	305	1024
Lincoln Bell	Green	Red	Late	4.2	-	-	64	678
Parks Early	Green	Red	Early	4.4	-	-	525	860
Staddons Select	Green	Red	Late	5.1	-	-	78	697
Stokes Early	Green	Red	Early	3.2	-	-	240	894
Super Set	Green	Red	Early	4.1	-	-	404	943
Super Stuff	Yellow	Orange	Early	3.7	205	560	168	780
Yellow Belle	Yellow	Red	Early	2.6	-	-	225	732
Yolo Wonder	Green	Red	Late	5.2	75	625	84	607

uncover the plants, and about $500/acre for frost protection.

Early yields required early planting as well as the application of row cover. When plants were transplanted late, and the row cover applied until July, production of ripe peppers did not begin any earlier than when peppers were planted late without row cover. Peppers planted early under row cover needed frost protection and the row cover did not provide this. However, sprinkling throughout the night when temperatures were below freezing or covering overnight with a second impermeable cover such as polyethylene film provided frost protection.

With these precautions, Connecticut farmers could produce an attractive crop of red or yellow peppers starting in late July and continuing until a killing frost.

Understanding behavior and ecology to reduce woodchuck damage

By Robert K. Swihart

The woodchuck, or groundhog as it is sometimes called, has become an integral part of American folklore because of its widely claimed skill as a winter weather forecaster. But while many cheer the antics of Punxsutawney Phil, farmers and backyard gardeners alike realize that Phil's shadow is a prelude to more than just a change in the weather. It signals renewal of the struggle to protect plants from damage by pesky woodchucks.

Woodchucks are widely distributed in North America, ranging as far south as Alabama and as far north as Alaska. In the continental United States they occur from the East Coast to the eastern edge of the Great Plains. Woodchucks belong to the squirrel family, and although they spend most of their time in underground burrows, they occasionally climb trees.

Throughout their range, woodchucks can pose problems for gardeners and farmers. When excavating burrows they typically deposit the soil near the burrow entrance, creating a sizeable mound. These mounds and holes can be hazardous to farm machinery as well as pedestrians. And if a burrow is excavated near a tree, excessive aeration of the tree's roots may result. Woodchucks also damage young trees by gnawing and clawing the main stem. And of course woodchucks are fond of crops such as lettuce, squash, pumpkins, beans, peas, melons, and tomatoes, as well as numerous types of flowers. Depending on the type of damage caused, growers may choose to remove woodchucks causing the problem, use fences to discourage entry into gardens, or apply chemical feeding deterrents to crops.

Although woodchucks are widely distributed and have damaged agricultural crops for decades, many aspects of woodchuck ecology and behavior remain unknown. Behavioral and ecological factors can influence the timing, extent, and type of damage inflicted. For instance, requirements for and availability of food and shelter, mobility, neighboring individuals, and the threat of predation all may directly influence damage, or they may act indirectly by altering the size of the pest population. By increasing our understanding of the reasons for damage, we may devise control strategies to the farmer's advantage.

Woodchucks spend much of their time in underground burrows. Most burrows have a main entrance characterized by a mound of excavated soil and one or more secondary entrances which are excavated from inside the burrow. Burrows consist of a network of tunnels that can be 10 yards long and extend to a depth of 5 feet. Woodchucks are choosy about where to construct a burrow; I found that they prefer to place burrows in well-drained, fine sandy loam soils and on moderately steep slopes. Burrows often were constructed in the strip of brushy habitat within 10 meters of orchards. At one orchard we found 8.7 burrows per acre (71 burrows) along the edge of the orchard, 1.5 per acre (48 burrows) in the woods, and 2.0 per acre (144 burrows) in the orchard. Furthermore, many of the edge burrows were associated with piles of brush.

How mobile are woodchucks? We monitored their movements using radio-telemetry and live-trapping. Both sexes are highly mobile, with adult males traversing 10 acres and adult females 7 acres. Furthermore, adult males used an average of eight different burrows within their home area, and females used an average of six. Although a burrow generally was occupied by one adult at a time, as many as six woodchucks used the same burrow during the summer and fall. In addition, individuals frequently moved back and forth between burrows in the orchard and burrows in the shrubby habitat along the orchard's edge. Over one fourth of all woodchucks located using telemetry were in edge burrows. The mobility of woodchucks and their habit of using numerous burrows may explain why control strategies aimed at removal of individuals from the population have met with variable success. In areas of high woodchuck density, if a woodchuck is removed, the burrow can be recolonized within an average of 5 days.

Burrows along the orchard edge are used frequently by females rearing young. By late May or early June, juvenile woodchucks have begun to feed almost exclusively on plant material. Woodchucks are mainly herbivorous but will eat some fruits. The availability and palatability of potential food plants has important implications for the amount of damage caused by woodchucks. Practices which encourage unpalatable plants may discourage use of a site by woodchucks.

A variety of ground cover plants that grow in orchards comprise the majority of the woodchucks' food supply there. We examined the preferences of woodchucks for 20 species of plants found in orchard ground covers in Connecticut. Plants were presented simultaneously to woodchucks, and the amount of each species consumed was recorded. Preferred plants were defined as those eaten more frequently than expected based on their availability, whereas neutral plants were eaten in proportion to their availability and avoided plants were eaten less frequently than expected based on their availability (Table 1). Woodchucks are generalist feeders; all 20 species were eaten to some degree. Nonetheless, woodchucks exhibited distinct preferences for some plants and avoided others (Table 1). Most notably, two species of grasses, redtop and orchard grass, were unpalatable. In contrast, two weedy species, dandelion and plantain, were the most highly preferred foods. Some differences in food preferences were evident between adults and juveniles. For instance, adults preferred poison ivy leaves, but juveniles avoided them.

How can feeding of woodchucks be reduced in vegetable and flower gardens? Fences, if properly constructed, can discourage woodchucks and other medium-sized mammals from entering a garden. The lower edge of wire mesh fences should be buried to a depth of at least 1 foot to discourage burrowing under the fence. The fence should extend 3 to 4 feet aboveground. Woodchucks are able climbers, thus it may be necessary to bend the top edge of the fence away from the garden, creating an overhang which the woodchuck cannot scale. Alternatively, a wide band of aluminum flashing can be fastened to the top of the fence. An electrified wire positioned 4-5 inches away from the fence and 4-5 inches aboveground will also help, provided that the area around the wire is kept free of vegetation.

If fencing is not feasible, other measures are needed to protect vegetable crops. Certain chemicals applied to plants deter animals from feeding because of taste or odor. However, most of these repellents are not registered for use on edible plants. Of those that can be used on vegetable crops, little is known about their effectiveness against woodchucks. We tested one such repellent, Hot Sauce, on squash plants. Untreated leaves of 1 month-old zucchini and acorn squash were presented to woodchucks, and feeding was monitored for 3 days. On average, 88% of the zucchini leaves and 94% of the acorn squash leaves were eaten each day. For the next 3 days the woodchucks were fed plants that had been sprayed with Hot Sauce. Consumption fell to 70% and 74% for zucchini and acorn squash, respectively. Although the Hot Sauce treatment reduced consumption, damage remained unacceptably high.

Repellents in general do not eliminate damage. Rather, they reduce damage by lowering the palatability of the treated plant. The magnitude of this reduction presumably is governed by the feeding pressure on the crop, which in turn is determined by the number of depredating animals and the availability and palatability of alternative foods. Thus, repellents may work better in the presence of alternative foods of relatively high palatability. We tested this hypothesis by simultaneously presenting leaves of butternut squash along with six species of plants commonly found in the woodchucks' natural habitat that were more palatable than squash leaves. The six naturally-occurring plants were dandelion, common plantain, mugwort, soapwort, red clover, and poison ivy. Consumption of each type of plant was monitored for 2 days. For the next 2 days the leaves of butternut squash were sprayed with Hinder, another repellent registered for use on vegetable crops. Average consumption of butternut squash dropped 68%, from 8.9 grams to 2.8 grams eaten per day. Hinder did not reduce food intake; rather, the woodchucks compensated for the reduced palatability of squash leaves by eating more of alternative plants such as soapwort.

Examining the ecology and behavior of woodchucks can, as shown, provide an increased understanding of the factors influencing damage as well as the timing and extent of damage. And an understanding of their feeding behavior may allow development of more effective feeding deterrents. I am continuing to work toward integrating this knowledge into safe, effective control strategies for woodchucks.

Table 1. Relative preferences of woodchucks for 20 species of plants commonly found as ground cover in orchards, in descending order of preference.

Preferred	Neutral	Avoided
Dandelion	Strawberry	Corn speedwell
Common plantain	Poison ivy	Rough-stemmed goldenrod
Mugwort	Common cinquefoil	Field hawkweed
Red clover	Cow vetch	Redtop
Small white aster	Blue grass	Orchard grass
White clover	Common mullein	Five-leaved ivy
Soapwort		Butter-and-eggs

Increasing photosynthesis by changing the genetics of leaf biochemistry

By Israel Zelitch

...carbon is the key element of living substance: but its promotion, its entry into the living world, is not easy and must follow an obligatory, intricate path, which has been clarified (and not yet definitely) only in recent years. If the elaboration of carbon were not a common daily occurrence, on the scale of billions of tons a week, wherever the green of a leaf appears, it would by full right deserve to be called a miracle.

-Primo Levi, "The Periodic Table" (Schocken Books, 1984)

The photosynthetic process by which plants use the energy of sunlight to convert to food an impurity in the air, consisting of only 0.035% (350 parts per million) [ppm] carbon dioxide, is usually taken for granted, although it is something of a miracle as poets and story tellers sometimes remind us. In the Department of Biochemistry and Genetics at the Experiment Station, scientists who specialize in biochemistry, plant physiology, and genetics are seeking new ways of enhancing this miracle by genetically changing the biochemistry of leaves.

Farmers, gardeners, and even growers of houseplants try to maximize photosynthesis by their actions in planting, fertilizing, watering, and caring for plants. Although often not aware that their activities affect photosynthesis, they all observe with fascination the sequences of plant growth and development which are wholly dependent on photosynthesis. They share a creative sense of accomplishment when they produce bigger and better plants and yields.

Drought and the adverse effects of water stress on photosynthesis and its association with famine are well recognized by everyone. But few people are aware of the constant "oxygen stress" on photosynthesis encountered by most plants. I will discuss research being done to decrease this oxygen (O_2) stress and thereby increase photosynthesis.

Air contains 21% O_2, which is of course needed for life, but ironically this amount is toxic for most plant species because it inhibits photosynthesis 33-50%. If such plants could be easily grown in a closed system in an atmosphere that contained only 1% O_2, growth and yields would be greater without any other inputs. Plants can therefore be considered always to be suffering from O_2 stress when they are grown in air. The extent of O_2 stress in such plants depends on the O_2 concentration as well as on the amount of carbon dioxide (CO_2) in the air. The greater the CO_2 at a fixed level of O_2, the less the O_2 stress. Although plants cannot easily be grown in 1% O_2 atmospheres except in a laboratory, it is not difficult to raise the CO_2 concentration four-fold in a closed system such as a greenhouse in winter. This increases photosynthesis, in part by alleviating some O_2 stress, and is a practical way of obtaining increased yields of greenhouse crops but not field crops.

I found that if tobacco seedlings are grown in the laboratory in double the normal O_2 concentration (42%) at still lower than normal CO_2 levels (160 ppm), the plants become yellow and sickly and many died within several weeks because of excessive O_2 stress. If the amount of CO_2 is raised far above normal in 42% O_2 the plants grow just as well as they do in 21% O_2. This is shown in Table 1 where plant growth over a 7 day period was measured in a constant environment as the increase in dry weight per unit of leaf area per day. Such experiments demonstrating reversibility of O_2 toxicity show that the harmful effects on growth in 42% O_2 and low CO_2 are associated with a sequence of biochemical reactions whose importance in the leaf increases as the ratio of O_2:CO_2 is raised. The sequence produces CO_2 and only in the light; therefore it is called photorespiration ("light respiration"). Since photorespiration is associated with that part of O_2 stress that is completely reversed by raising the CO_2 level, plantlets were screened in 42% O_2 to identify genetically altered plants that grow well under such conditions because they have decreased photorespiration.

Using plant tissue culture to increase the frequency of variants, tobacco plantlets were obtained with half the usual number of chromosomes. In the selection system, 2,714 plantlets were screened for growth in 42% O_2. Of these, 26 plantlets grew in 42% O_2 and were classified as O_2 resistant, and 5 (about 1 in 500) showed O_2-resistant photosynthesis. As shown in Table 2, compared to normal plants at 300 ppm CO_2 at 30 C (86 F), these selected plants had similar rates of photosynthesis in 1% O_2, about 17% greater in normal air (21% O_2), and about 36% greater in 42% O_2. This novel characteristic of an increasing relative photosynthesis on raising the O_2 levels from 1% to 21% to 42% O_2 was used to identify plants with O_2-resistant photosynthesis when selecting plants for self-pollination.

Table 1. Effect of 42% O_2 and changing CO_2 on growth of tobacco seedlings. Numbers followed by the same letter are not different statistically.

O_2 Conc.	CO_2 Conc.	Average Daily Growth Rate
%	ppm	mg wt increase per dm ^{2} leaf
21	160	29.9[a]
42	160	18.3[c]
21	10,000	72.1[b]
42	10,000	79.3[b]

To study the biochemistry and genetics of such O_2-resistant photosynthesis, fertile plants were needed and plants with half the number of chromosomes are infertile. The lateral buds of one O_2-resistant plantlet were treated with a chemical that doubles the chromosome number, and in this way shoots were obtained that contained fertile flowers that could be self-pollinated. The progeny of this first selfing produced a population in which 6 of 19 plants tested (about 30%) had O_2-resistant photosynthesis similar to that shown in Table 2. When one of these O_2-resistant plants was self-pollinated, the progeny of the second selfing contained 6 of 12 plants (50%) with O_2-resistant photosynthesis. This behavior is typical of traits that may require a number of successive self-pollinations to obtain a homogeneous population.

Table 2. Effect of O_2 concentration on net photosynthesis of typical O_2-resistant and normal tobacco seedlings.

O_2 Conc.	Average Net Photosynthesis of Leaves: O_2-Resistant	Normal	Ratio of O_2-Resistant: Normal
%	mg CO_2 uptake per dm ^{2} leaf/hour		
1	22.3	22.4	1.00
21	12.0	10.3	1.17
42	6.15	4.51	1.36

Experiments have begun to identify the biochemical changes associated with O_2-resistance. One line of investigation that shows promise is based on the idea that photorespiration increases greatly with increasing O_2, and incidentally also with increasing temperature, because compounds known as ketoacids are easily attacked by hydrogen peroxide generated by reactions occurring during photorespiration. Such a breakdown of ketoacids would generate additional photorespiratory CO_2. Ordinarily hydrogen peroxide is itself rapidly and effectively decomposed with the aid of an enzyme called catalase, a protein catalyst present in leaves. We reasoned in part based on work done earlier by colleagues Kenneth Hanson and Richard Peterson that perhaps when photorespiration rates are greatly increased, as occurs at higher O_2 and temperatures, there is insufficient catalase activity and excessive CO_2 production results from the breakdown of ketoacids.

In all comparisons we have made, the catalase activity of O_2-resistant leaves was greater than for normal leaves, and the increase averaged a highly significant 40%. Sibling plants with normal catalase activity did not show O_2-resistant photosynthesis. When photosynthesis was compared at 30 C (86 F) and 38 C (100 F), O_2-resistant plants were still better relative to the normal plants at the higher temperature. These results suggest that these O_2-resistant plants are superior under these conditions because overproduction of catalase helps prevent loss of CO_2 and thus brings about an improvement in net CO_2 uptake. Further experiments should help pinpoint the exact biochemical nature of this kind of O_2-resistance. We shall then know whether overproduction of catalase can be used as an indicator of increased net photosynthesis and crop yields especially at higher temperatures, and whether we can improve the efficiency of the miracle of photosynthesis in such a rational way.

Composted sewage sludge and leaves make desirable potting media

By Gregory J. Bugbee

Environmentally and economically acceptable methods of waste disposal are becoming increasingly scarce. As one consequence, the Connecticut General Assembly has mandated that each town have a plan for recycling 25% of its waste by 1991. Hence, many towns are considering composting of organic wastes such as leaves, woodchips, and sewage sludge that can then be recycled as a soil amendment.

Composting involves piling the waste, supplying adequate air and moisture, and allowing naturally occurring microorganisms to create high temperatures which enhance decomposition. Depending on the sophistication of the technique, a dark, humus-like material, called finished compost, is produced in from several weeks to several years. Finished compost usually has a near neutral pH, high plant nutrients, no offensive odors, and no pathogens considered harmful to humans or plants.

Large-scale composting of wastes is not new to Connecticut. A nursery in Darien has composted municipal leaves for over 20 years. Greenwich has composted its leaves, sewage sludge, and sand from street sweepings and catch basins for about 10 years. Recently, a variety of wastes are being composted with in-vessel techniques by a private firm in Lebanon.

Ornamental nursery crops are among Connecticut's most valuable agricultural commodities. Presently, large proportions of the crops are grown in containers filled with relatively costly media. A quality medium must be high in plant nutrients, have good aeration, hold large quantities of water and be free of pathogens and weed seeds. To produce this, growers often purchase components such as peat moss, topsoil, sand, and Styrofoam pellets, which are then laboriously mixed with fertilizer and limestone and pasteurized by adding steam or a soil fumigant. If composted waste could replace some of the conventional components, producers of both compost and nursery crops could benefit.

With the cooperation of Sunny Border Nurseries in Kensington, I examined whether municipal compost could be substituted for the components in a conventional medium used to grow perennial flowers commercially. Compost made from 66% (by volume) leaves, 16.5% digested sewage sludge, and 16.5% street sweeping and catch basin sand was obtained from Greenwich in April 1988. The materials had been composted by the Beltsville aerated pile technique, which entailed piling in the fall of 1987, aerating during the winter, and screening in early spring. After screening the compost is ready for use.

Conventional media containing soil, Canadian peat, sand, and Styrofoam (Table 1) was amended with compost to form five experimental media. Except in the case of the medium containing 30% peat, compost was added to completely replace a component and therefore simplify the media. Granular fertilizer was added at a conventional rate of 1 lb (per cubic yard) nitrogen, 0.5 lbs phosphorus and 0.5 lbs potassium to all media and limestone was added to the peat to raise its pH to 6.5. In late May rooted cuttings of the perennial flowers Sedum "Autumn Joy," Aster "Peter Harrison" and Guara lindheimeri were placed in 2-liter containers filled with each medium. After 3 weeks in a cold frame the containers were arranged in the field in randomized blocks.

Table 1. Media components.

Compost	Soil	Peat	Sand	Styrofoam
		(% vol)		
0	10	50	20	20
10	0	50	20	20
30	0	30	20	20
60	0	0	20	20
80	0	0	0	20
100	0	0	0	0

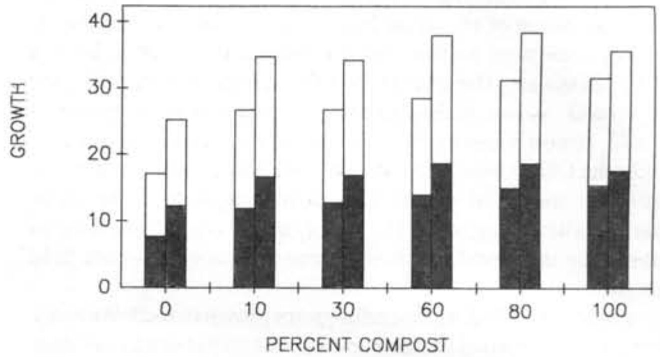

Figure 1. Growth of Sedum in compost from Greenwich. The dark bars show growth of flowers and the light bars show growth of stems and leaves. At each % of compost, the bars on the left show the effect of no fertilizer and the bars on the right show the effect of liquid fertilizer.

Because composts can contribute additional plant nutrients to media, I also determined if a typical application of liquid fertilizer during the growing period was needed. Five replicates of each medium received 300 ml of liquid fertilizer containing 400 ppm N, 88 ppm P and 332 ppm K in late July while an additional five did not. In early October, aerial plant parts were harvested. To determine if flowering was affected by adding compost, flowers were separated from the stems and leaves. Previous studies indicate that increased plant quality is related to increased dry weight of plant parts. Thus, after being dried the weights of the flowers and the combined stems and leaves were obtained. Figure 1 illustrates how the mean dry weights of Sedum were affected by the addition of compost to the media. Aster and Guara responded similarly.

In all media containing compost, plant growth was equal or better than in the conventional medium. Liquid fertilizer stimulated growth in all media, but plants grown in 100% compost without liquid fertilizer grew about as well as plants in conventional media with liquid fertilizer. In no case did flower production decrease when leaf and stem production increased.

All media were analyzed for major plant nutrients at monthly intervals during the growing season. Results suggested that increased nutrient levels, especially nitrate nitrogen, were responsible for much of the improved growth observed in media containing compost. Tests on the physical media properties showed compost improved aeration but reduced water holding capacity. Although better aeration could have improved plant growth, the reduction in water did not likely limit growth since the plants were irrigated regularly. A commercial grower may notice an increased need for irrigating crops grown in compost, but this should not negate its benefits.