FRONTIERS of Plant Science

SPRING 1996
Volume 48 No. 2

Chris T. Maier checks for periodical cicadas

Growing vegetables in compost-amended soils

Periodical cicadas are returning

Cloning corn genes for crop improvement

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION,

founded in 1875, is the first experiment station in America. It is chartered by the General Assembly as an independent State agency governed by a Board of Control. Station scientists make inquiries and experiments regarding plants and their pests, insects, soil and water quality, food safety, and perform analyses for State agencies. Factual information relating to the environment and agriculture is provided freely and objectively to all. The laboratories of the Station are in New Haven and Windsor; its Lockwood Farm is in Hamden. Copies of this and other publications are available upon request to Publications; Box 1106; New Haven, Connecticut 06504

ISSN 0016-2167

Growing vegetables in soil amended with compost or leaves

By Abigail A. Maynard

The shortage of acceptable landfill sites has made the disposal of municipal and agricultural wastes an increasingly difficult problem in Connecticut. To reduce the impact on current landfill sites, the state enacted a law in 1991 which stipulates that 25% of all wastes must be recycled and lists materials no longer accepted at landfills. The inclusion of leaves on this list has caused municipalities to examine composting as an alternative for waste management.

Many municipalities, without suitable land to compost leaves, approach local farmers to use their land. While many agree to accept leaves, some farmers are unwilling to tie up agricultural land for a year or more or to expend the labor and capital for a composting operation. These farmers would prefer to plow under a layer of undecomposed leaves applied directly to their field and plant their crops in the leaf-amended soil (sheet composting). As leaves decompose in the soil, however, nitrogen may become limiting, and might decrease yields. The objective of this study was to determine the suitability of annual applications of undecomposed leaves as a soil amendment for vegetable production.

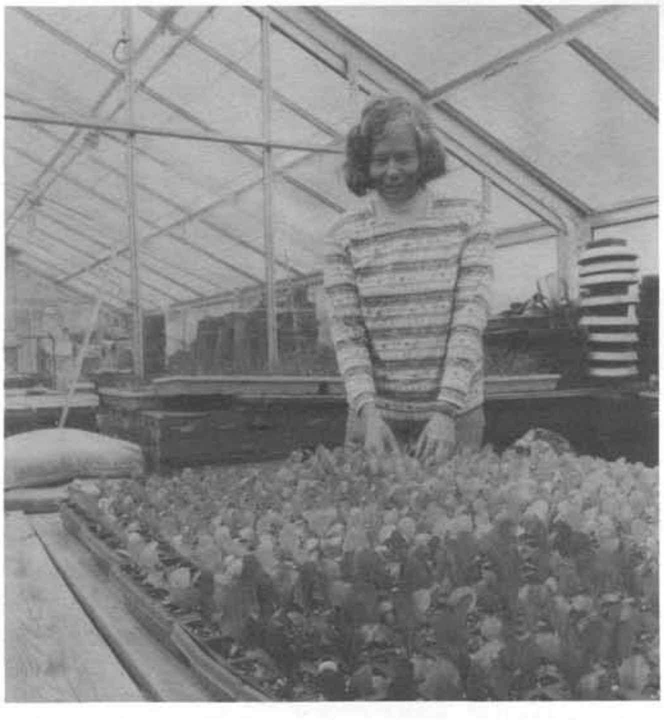

Figure 1. Abigail A. Maynard checks lettuce seedlings being grown for compost experiments.

The trials were conducted at the Valley Laboratory in Windsor on a sandy terrace soil with a somewhat limited moisture holding capacity and at Lockwood Farm in Mt. Carmel on a loamy upland soil with a moderate moisture holding capacity.

Each plot was 20 X 20 feet separated by 3-foot aisles and replicated four times. Six inches of undecomposed leaves were applied to one set of plots in November 1991, 1992, and 1993 (fall-applied leaves) and another set in April 1992, 1993, and 1994 (spring-applied leaves). The leaves were incorporated into the soil by rotary tilling in two directions, perpendicular to one another. Yields from these plots were compared to yields in plots amended in April 1992, 1993, 1994 with 1 inch (50 tons/acre) of finished leaf compost and unamended controls. The leaf compost was produced in a passive pile turned two to three times yearly. All plots were fertilized with 10-10-10 at a rate of 1300 lb/A (12 lbs/plot).

Eight crops were grown: broccoli, cauliflower, and lettuce in the spring; tomatoes, eggplant, and peppers in the summer; and broccoli and cauliflower in the fall. I am reporting results from the mid-season crops: tomatoes, eggplant, and peppers grown at Mt. Carmel. Yields from Windsor followed the same trends.

Each year, the crops were seeded in the greenhouse at the end of March. They were transplanted in the field at the end of May in rows 3 feet apart with a spacing of 2 feet within the rows for eggplant and tomatoes (10 plants/plot) and 1.5 feet within the rows for peppers (13 plants/plot). Vegetative suckers on the tomatoes were removed up to the first flower cluster and the plants staked. Marketable fruit were harvested weekly until frost. Weeds were controlled by cultivation. Plants were removed from all plots at the end of each growing season. Soil samples were obtained from each plot in October at the end of harvest.

For all 3 years at Mt. Carmel, the greatest yields of eggplant were from plots amended with leaf compost and the smallest yields were from plots amended with undecomposed leaves in the fall (Table 1). The difference in yield was statistically significant in all years. Compared to the unamended control, yields from plots amended with leaves in the fall were reduced 11%, 20%, and 19% in 1992, 1993, and 1994, respectively. Reduction in yield began early in the growing season in all 3 years.

The greatest yields of peppers in all 3 years were from plots amended with compost and the difference was significant in 1993 and 1994 (Table 1). Plots amended with leaves

This study shows that the greatest eggplant and pepper yields are obtained when the soil is amended with fully-mature leaf compost. If compost is not available, spring application of leaves that have been stockpiled over winter provides a material that is not phytotoxic. Consistently high tomato yields are also obtained from plots amended with leaf compost but applications of undecomposed leaves in fall or spring are not detrimental to yield. The reason why yield of tomatoes, unlike eggplant and peppers, was not reduced from fall-applied leaves is not fully understood.

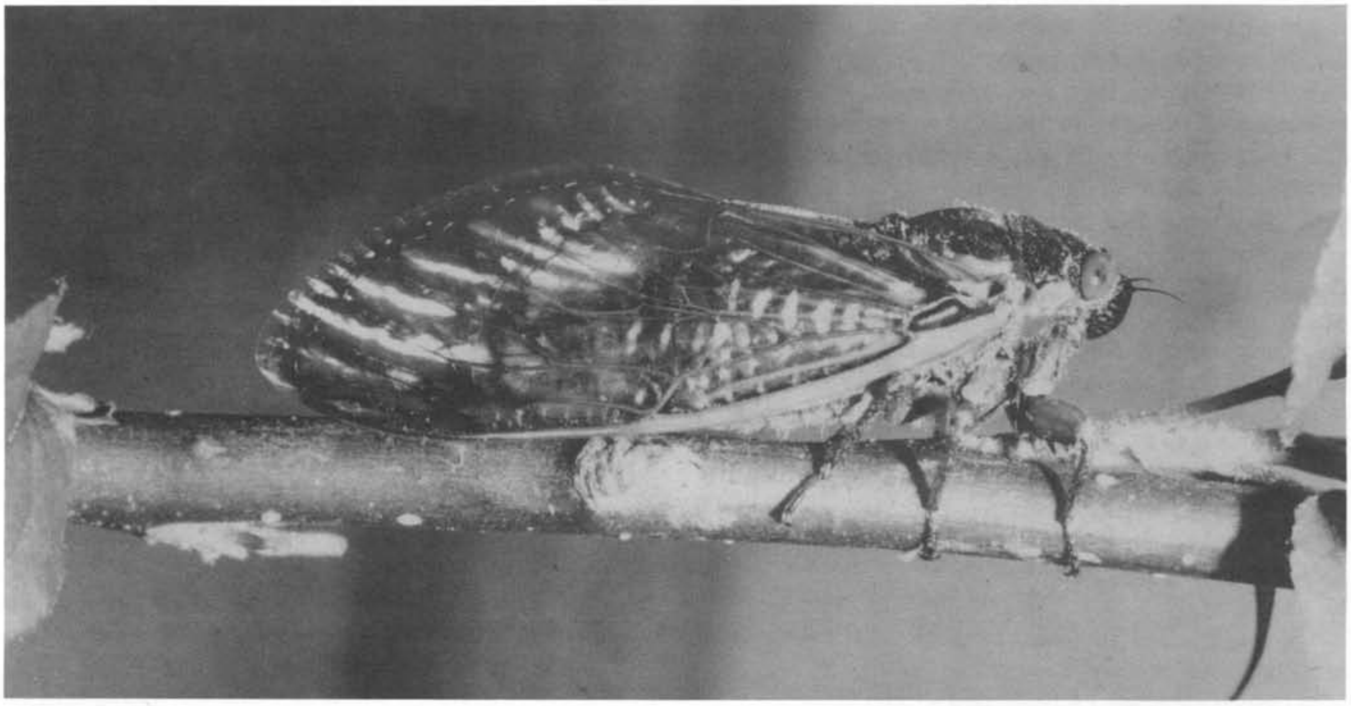

Figure 1. Adult of the 17-year periodical cicada.

Connecticut is awaiting return of the periodical cicada

By Chris T. Maier

The woods of Connecticut will soon be alive with sound. This spring adults of the 17-year periodical cicada (Fig. 1), last seen in 1979, will again emerge in the forests and the orchards of Connecticut. For centuries, the mass emergences of these noisy insects have delighted and amazed scientists and laymen alike.

Periodical cicadas have the longest development cycle of any insect. Nymphs (Fig. 2) slowly develop underground by feeding upon tree rootlets with their piercing-sucking mouthparts. The xylem fluid in rootlets is so nutritionally poor that some scientists have suggested that this diet is responsible for their lengthy developmental period of almost 17 years.

Since the last emergence, I have sampled nymphs annually in a Southington forest to determine how they grow. The nymphs have five instars (the nymphal stage between successive molts is called an instar). First instars were most abundant in 1979, second instars between 1980 and 1982, third instars between 1983 and 1986, fourth instars between 1987 and 1990, and fifth instars between 1991 and 1995 (Fig. 3). In the autumn of 1995, the eye color of nymphs changed from white to red, signaling their emergence in the following spring.

Early in the evening in late May and June of this year, the long ordeal of nymphs will end when they emerge from the soil, climb the trunks of trees, and molt to the adult stage. The adults must reproduce during their short life of 2-3 weeks. Males attract mates by singing. Their characteristic song is produced by rapidly vibrating a pair of ribbed tymbals located at the base of the abdomen. Their call for mates is hard to ignore because males sometimes chorus by the thousands or even the millions. Imagine the sound pro-

Figure 2. Full-grown fifth-instar nymph.

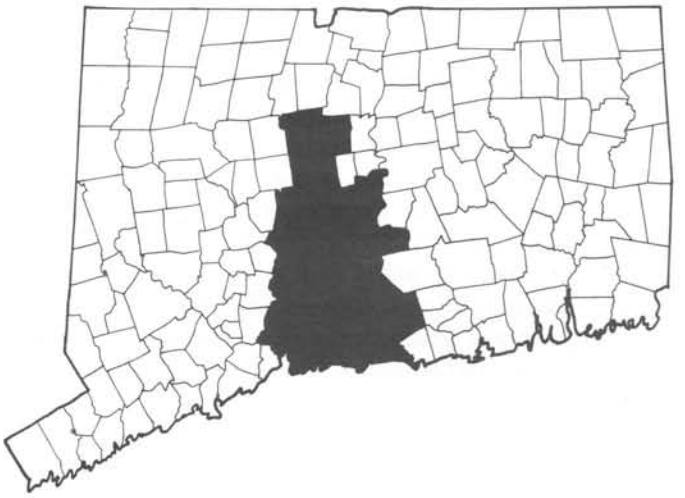

Figure 4. Distribution of Brood II of the 17-year periodical cicada in Connecticut. All towns in black had cicadas in one or more past emergences.

duced by one dog-day cicada in summer, and multiply that sound by a thousand or a million. The clamor is not soon to be forgotten.

All 17-year periodical cicadas that emerge in the same year are assigned to a numbered brood. In Connecticut, the only extant brood is Brood II. This brood is restricted to central and south-central Connecticut (Fig. 4). The first Connecticut specimens of Brood II were collected near West Rock in 1843 by C.L. Hillhouse.

Historically, Brood XI occurred in the Connecticut towns of Suffield and Willington. In a letter sent to an entomologist at the USDA, George Dimmock wrote this about Brood XI in Suffield: *When I saw them in 1869 the cicadas were so abundant that small bushes and undergrowth in the rather sparse woods in which they occurred were weighted down with them.* The last adults of Brood XI were seen in Willington in 1954. The demise of Brood XI was hastened by cutting of forests as land was developed for use by humans.

Three species of 17-year periodical cicadas inhabit the Northeast, but only the most common species, *Magicicada septendecim*, occurs in Connecticut. In 1979, the adult density in deciduous forests was estimated at 8,900/acre in Guilford, 24,700/acre in Middlefield, and 32,400/acre in Southington. How much the size of populations fluctuates from emergence to emergence is unknown. In 1996, I will determine where cicadas still occur and how abundant they are.

Six species of cicadas are found in Connecticut. In addition to the one periodical cicada, there are five species of dog-day cicadas in the genus *Tibicen*. One species, *Tibicen*

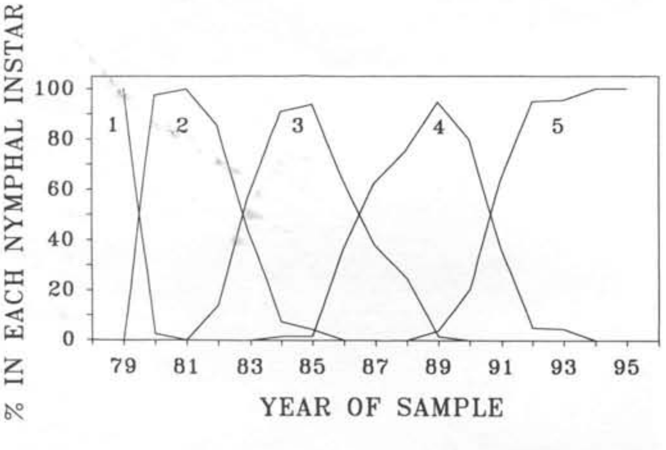

Figure 3. Percentage of each nymphal instar found in forest soil sampled annually in Southington between August-November of 1979-95. The number under each line indicates the nymphal instar.

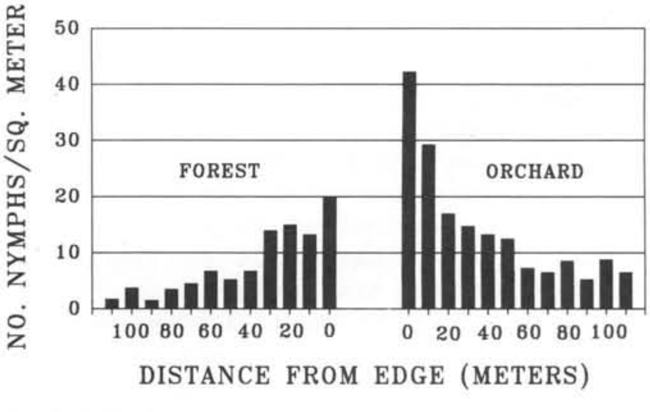

Figure 5. Mean density of the 17-year periodical cicada along four straight-line transects perpendicular to the junction of an apple orchard and a forest in Southington in 1979. Densities were based on counting the emergence holes of nymphs.

auletes, was found in the New Haven area in the 1910's by the eminent Station entomologist, Wilton E. Britton, after whom the Britton Building is named. This dog-day cicada has not been seen since and is now listed as a "Species of Special Concern" by the Department of Environmental Protection. The bodies of adult cicadas are one to one and one-half inches long. Periodical cicadas are black with red or reddish orange markings. By contrast, dog-day cicadas are mainly green and black. Periodicals appear mainly in late May and June, whereas dog-day cicadas are active from July to the first frost.

Although many consider the mass emergence of periodical cicadas to be a wonderful natural phenomenon, fruit-growers have a different perspective. Adult females can injure fruit trees when they lay eggs. They cut a slit in small branches with their sawlike ovipositor before they deposit eggs in a nest carved in the wood. The twigs dry out where the eggs were laid, and then often die and break. If damage is extensive, young trees can be disfigured. Sometimes orchardists must apply insecticides to protect their trees. Such sprays can be directed to the orchard periphery where the adults are concentrated (Fig. 5).

By early July, the clamor will be over. The only evidence of the mass emergence will be the cast skins of nymphs, dead bodies of a few adults, and dead twigs and leaves on trees—and these, too, will soon disappear. In late summer, the nymphs will hatch from eggs and burrow into the ground to begin another 17-year cycle. Periodical cicadas will then be out-of-sight and out-of-mind until 2013 when the woods, again, will reverberate with their sound.

Cloning corn genes today for future crop improvements

By Neil P. Schultes

Genes are now directly manipulated through biotechnology in the laboratory and reintroduced into plants. The resulting 'transgenic' plants hold great promise for crop improvement. Benefits from genetically-engineered crops include the use of environmentally-friendly biopesticides to replace and reduce our current reliance on harmful insecticides and fungicides. Manipulating plant disease resistance genes offers a sophisticated layer of defense to crops continually assaulted by disease organisms. In addition, altering plant genes involved in photosynthesis will increase crop yields, while modifying drought response genes will allow marginal land to be economically farmed.

The small number of already approved transgenic plants and many of the genetically-engineered plants now under review contain single gene changes or additions and represent only the first wave of this new technology (e.g. Calgene's Flavr Savr tomato and Mycogen and Ciba Seeds' *Bacillus thuringiensis* biopesticide-producing corn for insect resistance). However, to manipulate complex plant processes such as photosynthesis, future genetically-engineered plants will require multiple gene changes. Therefore, identifying and understanding key genes in complex plant processes today will allow successful genetic engineering in the future.

I am collaborating with Drs. Steven Dellaporta and Timothy Nelson at Yale University, combining classical genetics in the field with modern molecular techniques in the laboratory to identify photosynthetic genes in corn. Corn is one of a few crop plants that performs C4 photosynthesis—a specialized variation of photosynthesis resulting in efficient use of light energy for carbon dioxide fixation and sugar production. The combination of 90 years of genetic

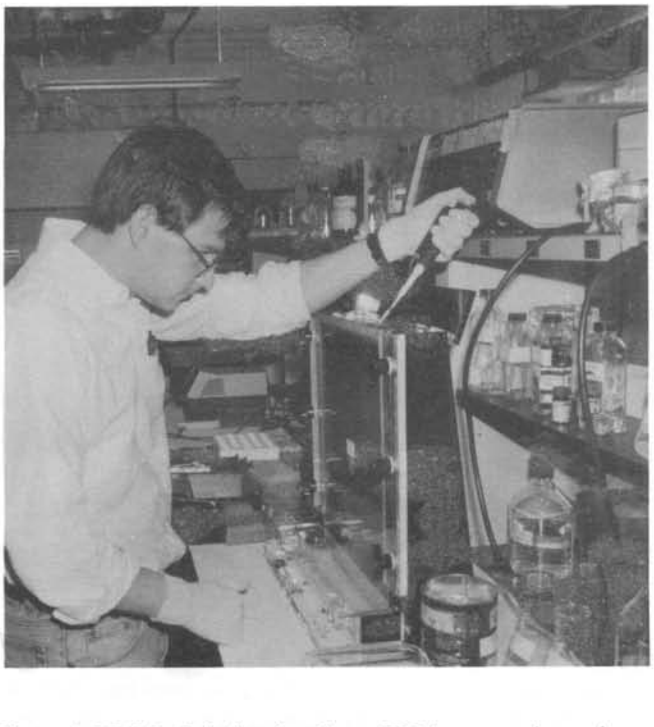

Figure 1. Neil P. Schultes loading a DNA sequencing gel.

Transposable Elements

Transposable elements are segments of DNA that move to new positions on chromosomes. The one or two genes present in the transposable element DNA encode proteins that facilitate excision of the element DNA from one position on a chromosome and aid in its reintegration into another site on chromosomes. Maize has many different transposable element systems; the most extensively studied are the *Ac* (*Ac*tivator), *Spm* (Suppresser) and *Mu* (*Mu*tator) systems. Indian corn, popular in seasonal home decoration, often contains variegated colored seed resulting from the movement of a transposable element out of a gene involved in seed pigmentation.

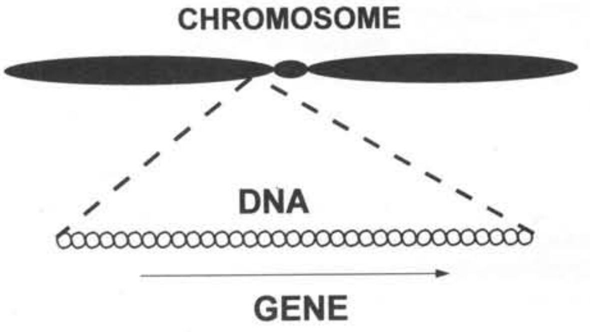

Figure 2. A chromosome is made of a long tightly wound DNA molecule. A gene (designated by the arrow) is a small segment of DNA that encodes for one protein.

research on corn and the exploitation of endogenous transposable element systems makes corn an ideal organism for such research. New photosynthetic genes are identified by generating mutations and then isolating the defective gene.

Genes are small segments of the long DNA molecule that makes up a chromosome (Fig. 2). Each gene encodes information to produce one protein, usually an enzyme, that facilitates one chemical reaction. Plant chromosomes contain an estimated 40,000 genes.

Genes are visualized through mutations and their effects. Genes act together to produce a normal plant (Fig. 3). A mutation in a gene inactivates the encoded enzyme causing a loss of function in the plant. This deficiency is often visible as an altered plant character. For example, plants with a mutant photosynthetic gene often have pale green or white leaves due to the loss of pigments.

Genetically-engineered plants

The Flavr Savr Tomato developed by Calgene is one example of a genetically-engineered organism. This transgenic tomato is delayed in ripening. A reversed or 'antisense' copy of the gene encoding the cell wall-degrading enzyme polygalacturonase was added to the plant DNA. The expression of this reversed gene slows the production of the cell wall-degrading enzyme resulting in a delayed ripening.

Mutations in corn can be generated by mobile pieces of corn DNA called transposable elements. These elements, such as the *Ac* (*Ac*tivator) element discovered in the 1940's, move to different locations in corn chromosomes. Occasionally an *Ac* element will insert into and disrupt a gene, causing a new mutation (Fig. 3). The resulting mutant plant is often a mosaic of mutant and normal tissue sectors. The mutant sectors contain a gene disrupted by the *Ac* element, while the normal sectors result from the excision of the *Ac* element from the mutated gene, thereby restoring the gene to normal activity. Genes disrupted by a transposable element are "tagged".

The search for new genes is split between field genetics and laboratory experiments. A mutagenesis screen requires 3 years of field genetics to complete one cycle. In the first season corn seed containing actively-moving *Ac* elements is generated, harvested and identified. During the second season these seeds are planted and the ensuing plants are self pollinated. All crosses are controlled, with the pollen from a tassel harvested and used to fertilize the eggs located on the cob of the same or a different plant. In the third season, resulting progeny are screened for mutant plants, particularly those with a mosaic character. These seeds are grown in sand benches in the greenhouse to reveal mutations at the seedling stage and later in the field to reveal mutations that occur in adult plants. Each summer approximately 3 acres at Lockwood Farm is devoted for each stage of the mutagenesis to generate a supply of mutant plants. Within the 3-4 week pollination period (usually starting at the end of July) about 3000 crosses are performed. For every 1000 mutant seed generated, 1-10 mosaic-appearing mutants arise, most caused by an *Ac* element insertion into a new gene.

The second phase of this research occurs in the laboratory where the mutated genes are cloned from mutant plants. Gene cloning, a months-long task, involves isolating a piece of DNA (usually carrying a gene) away from all other DNA in the cell and producing a workable amount of this DNA molecule (usually 100 µg). A gene library is composed of gene-sized fragments of DNA (from the mutant corn plant) encapsulated in a genetically engineered bacterial virus which serves as a vector or vehicle for amplifying small

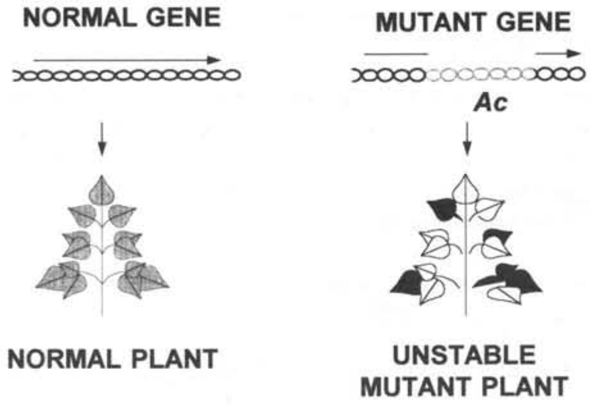

Figure 3. Genes work together to produce a normal plant. The insertion of *Ac* DNA (grey) into a gene causes a mutation and causes an unstable mutant plant.

DNA fragments. Approximately 200-500,000 such virus collectively contain all of the mutant corn DNA. The one virus containing the *Ac*-tagged corn gene DNA is found by using radioactivity labeled *Ac* DNA in a process called molecular hybridization. Once found, the virus containing the *Ac*-tagged corn gene is isolated and amplified to yield a workable quantity of the desired mutant gene DNA.

The availability of a cloned gene coupled with analysis of mutant plants lacking this gene's full function is a powerful combination for elucidating the gene's role. Several new genes involved in corn photosynthesis have been found. One such gene is called *leaf permease 1* (*lpe1*). DNA sequencing of the *lpe1* gene coupled with computer analysis reveals that this gene encodes a protein similar to bacterial proteins involved in molecule transport across cell membranes. Highly magnified pictures of *lpe1* mutant plant tissue through thin layer transmission electron microscopy reveal photosynthetic cells with disrupted chloroplast membranes. The chloroplasts in the cells are the site of CO_2 fixation in C4 photosynthesis and are composed of many membrane layers.

Physiological measurements of mutant corn tissue show that, although C4 photosynthesis still occurs, it is inefficient, presumably due to disruption in cellular molecular transport. Experiments are in progress to use this and other mutations as probes to investigate C4 photosynthesis.

The Experiment Station is
an Equal Opportunity/
Affirmative Action Employer

The Connecticut Agricultural
Experiment Station
Box 1106, New Haven, CT 06504