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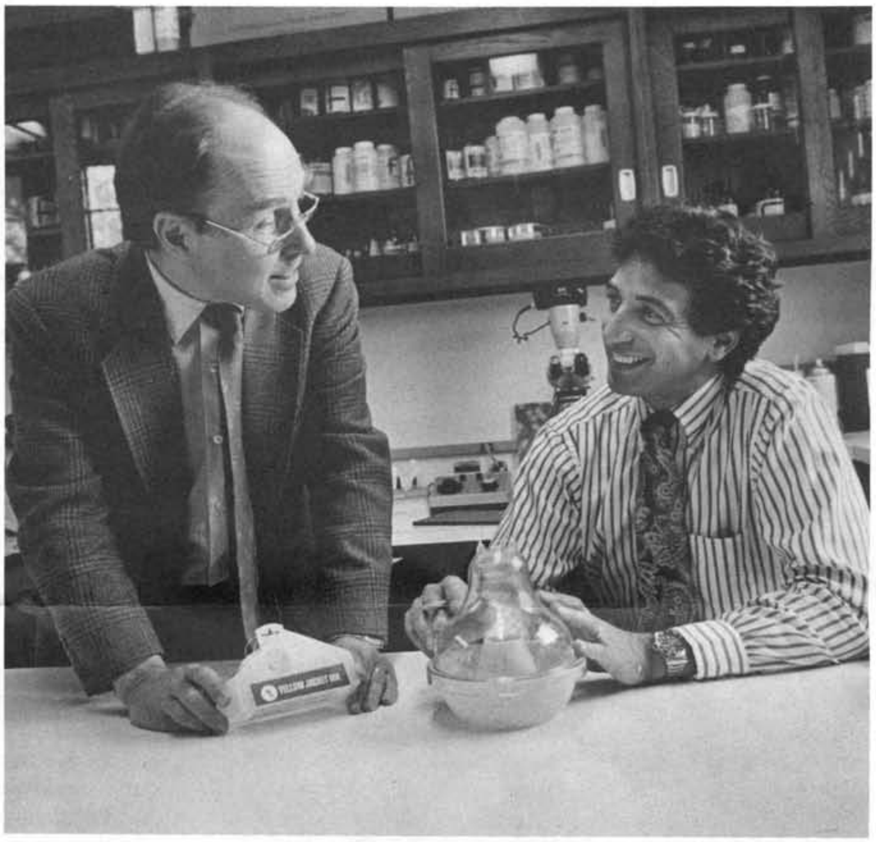

Kenneth A. Welch and Theodore Andreadis with wasp traps

Fresh-cut culinary herbs

Master genes for leaf development

Controlling yellowjackets and wasps

Taxol from ornamental yews

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

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Fresh-cut culinary herbs are easy to grow in Connecticut

By David E. Hill

Culinary herbs are mostly non-woody plants that contain essential (aromatic) oils that have been used for centuries as condiments to enrich the flavor and aroma of meat, fish, and vegetables. The earliest account of their use is from the Mycenean culture in the 13th Century BC. Cultivated herbs were spread throughout Europe by Roman legionnaires and ultimately found their way to America's shores at the turn of the 19th Century.

In Connecticut, the growing of herbs has long been practiced by ardent backyard hobbyists who employ them not only for culinary use but as ornamental plants in perennial gardens. In the past two decades, commercial production of potted and fresh-cut herbs has flourished among a few dedicated growers who report high cash returns for their efforts. Herbs were, therefore, worthy of study in the Station's "New Crops Program" whose objective is to provide growers with information on cultivars and their anticipated yields in Connecticut's soil and climate. There are many herbs that have culinary uses and the representative annuals and perennials that I chose for study are among the most popular. In this report, I will tell you about their yields and management in trials conducted between 1989 and 1991 at Lockwood Farm, Hamden.

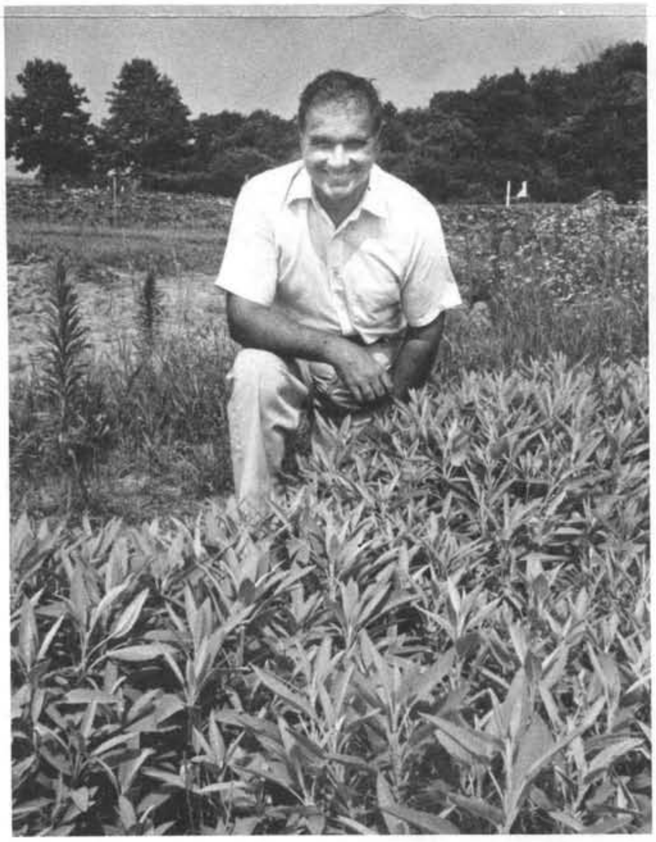

Figure 1. David E. Hill with sage growing at Lockwood Farm.

PARSLEY. Parsley is the most widely grown herb in the United States. Although parsley is not consumed in great quantities, its nutritional value cannot be overlooked. The Beta-carotene content (11 mg/100g) exceeds that of carrots. There are two types of parsley: the curled variety, used mainly as a garnish, and the plain or Italian broad-leaved variety, used mainly in cooking. Both types can be eaten fresh or quickly dried to preserve their green color and flavor. In early March, I sowed seeds of all herbs in a greenhouse in 36-pot packs filled with ProMix BX. I transplanted the seedlings in early May in soil fertilized with 10-10-10 fertilizer at a rate of 1300 lbs/A. The parsley was harvested periodically throughout the year by cutting the entire plant about 2 inches above the ground. Since parsley needs ample water and nitrogen to promote rapid vegetative growth, I side dressed the crop with ammonium nitrate (90 lb/A) after the first and third cuttings.

The average yield/plant of all cultivars of curled parsley was 14.9 oz and 22.1 oz for four cuttings in 1989 and 1990 respectively; and 11.0 oz in 1991, limited to three cuttings because of spring drought. Afro, Bravour, Sherwood, and Unicurl exceeded the average in at least 2 of 3 years. Yields of Darki and Forest Green were below average in all years. Among the cultivars of plain parsley, the yields of Gigante were greater than that of Plain Italian in 2 of 3 years, attaining 32.4 oz/plant in 1990 in four pickings.

FLORENCE FENNEL. Florence fennel, also known as finocchio, or sweet anise, is commonly grown as a vegetable, but its feathery leaves are often used to add a licorice flavor to salads. Nutritionally, fennel has a high vitamin C and free amino acid content compared to many herbs. The stalks overlap at the base to form a bulb. If blanching is desired, soil can be mounded about the base when the bulb is a diameter of 2 inches. Bulbs are harvested before the flowers form to prevent toughness. Fennel can be grown as a spring or a fall crop. Fall crops are less prone to flowering if planted in early July. Late July plantings may produce smaller bulbs whose growth is curtailed by frost.

In my trials of spring planted fennel, Zefa Fino, a hybrid cultivar, had greater yields than Florence Fennel, an open pollinated cultivar. In 1989, bulbs of Zefa Fino were 20.9 oz/plant compared to 6.5 oz/plant in 1991 with a droughty spring. In 1991, Fennel Fino and Romy Fennel had larger bulbs than Florence Fennel.

DILL. Dill is popular to grow and has many culinary uses. Most dill is grown by direct seeding in garden soil because it grows poorly from transplants. I have found, however, that transplants can be successful if grown in small transplant packs filled with potting mix and the plants are set with roots undisturbed. Dill leaves are first harvested when the plants are about 8 weeks old. Seed heads (umbels) are also harvested in the blossom stage for use in pickling. Seeds are harvested when they turn light brown. In commercial production, beds of fresh dill are thickly sown and harvested when the plants are

6 to 8 inches tall with roots intact. In 1990, yield was 1.7 oz/plant harvested from plants whose umbels were first forming.

SUMMER SAVORY. Summer savory is often called the bean herb because it complements green beans, dried beans, and lentils. It is also used extensively in herbal teas. Savory was started in a greenhouse and transplanted to a 10-12 inch spacing. Early harvesting, when the plants are about 6 inches high, is desirable because it delays flowering and promotes vegetative growth. The whole plant is harvested when flowers begin to form. Yields are highly dependent upon the moisture supply throughout the growing season. I harvested 17 oz/plant in 1990, a year of ample rain. In 1991, following spring and early summer drought, the yield was only 3 oz/plant.

MARJORAM AND OREGANO. Marjoram and oregano are related botanically, but oregano is winter hardy and can be grown as a perennial. In my trials, marjoram did not overwinter even when protected by mulch. Both can be grown from seed, cuttings, or crown divisions. Marjoram can be dug in the fall, grown indoors in winter and replanted in spring. Leaves are harvested throughout the growing season. When flowers first form, the plants are cut back to 4 inches which stimulates new growth. The second growth is the main crop. In my trials I harvested 8 oz/plant of oregano and 4 oz/plant of marjoram in 1991. Harvest in 1990 was intentionally light in the first year to permit adequate plant size for overwintering. Both have a variety of uses in green vegetables, salads, soups, and meat and egg dishes.

SAGE. Sage is a shrubby plant that grows 2-3 feet tall and overwinters well. In 3 years, only one of fifty plants was lost to winterkill. Sage may be grown from seeds, cuttings or crown divisions. Severe spring pruning of woody growth encourages vegetative growth and discourages flowering. The top 6-8 inches of new growth is harvested twice during the growing season. In my trials I harvested 3 oz/plant in 1990 as the new plants, grown from seed, became established and 6 oz/plant in 1991 from fully grown plants. Sage has a wide variety of culinary uses.

TARRAGON. There are three types of tarragon, French, German, and Russian. I grew the French type which is most highly prized for culinary use. Tarragon is mostly propagated from cuttings or root crown divisions. Its seed is seldom viable. Tarragon is somewhat susceptible to winter injury and should be mulched. It can be harvested throughout the growing season by cutting the top growth to promote branching. In my trials, I harvested 1-2 oz/plant in 1990 and 1991. It is extensively used in soups and sauces (Bernaise and tartar). Tarragon vinegar, made with wine or cider vinegar, is popular as a salad dressing.

THYME. Thyme can be started from transplants and cuttings. Dividing the plants every 3 to 4 years prevents woodiness. The spring harvest occurs just before the pink flowers appear. The plants are cut to 2 inches above the ground. A second crop is harvested in late summer, but one-third of the vegetation is allowed to remain to allow sufficient strength for overwintering aided by mulch. In my trials I harvested 7 oz/plant in 1991 following a light harvest in 1990 as the plants became established. Thyme is used with a variety of meat and vegetable dishes.

Essentially all the herbs in my trials were easy to grow in well-drained soil. Since the edible portion is mostly vegetative growth, they all need ample water, sunlight, and nitrogen supplied by annual applications of fertilizer.

Growing herbs is rewarding, not only for the labor of producing them, but also for the flavors they impart to your favorite foods.

Master genes for leaf development will provide tools for genetic engineering

By Neil A. McHale

The process by which a whole plant arises from a single embryonic cell has been an endless source of fascination to biologists and people who grow plants. The initial divisions of the embryo produce an amorphous mass of dividing cells, but, gradually, the cells at opposite poles are channeled into the separate programs of shoot and root development.

How are the patterns of cell division controlled with such precision? How does the embryo generate such a diversity of cell types? How do cells in such close physical proximity maintain distinct programs of development? How can we use this knowledge to improve plants? The answers to such questions had remained elusive for decades. But, with the advent of techniques for gene cloning and molecular analysis, these mysteries are unfolding.

Different regions of the embryo give rise to different parts because they are expressing different genes. Genes acting as master regulators of cell division have now been identified as the main switches controlling pattern formation in plants and animals.

In animals, the body plan and vital organs are fully established at birth. Plant embryos (seeds) contain the rudiments of the body plan, but new organs are formed throughout the life cycle by groups of rapidly dividing cells (meristems) located at the apex of the developing roots and at the summit of the shoot.

New leaves are formed by the shoot apex in a repetitive spiral or in alternating pairs, depending on the species. In the reproductive phase of growth, the pattern of cell division at the shoot apex shifts from leaf initiation to flower initiation. Shoot growth is normally terminated with the development of flowers and fruit. Until recently, the mechanisms controlling these basic aspects of plant development were unknown. But the techniques of molecular biology are beginning to uncover the master genes of the plant kingdom, and they are expected to have a broad impact in genetic engineering of agricultural plants.

Genetic manipulation of plant development will have many applications in commercial agriculture. Perhaps the most obvious involves control over flower and fruit development. The shoot apex initially produces only leaves in the vegetative phase of growth, but in response to environmental signals, such as photoperiod and temperature, the shoot starts producing flowers. Since flowers often develop into the edible part of the plant (fruit), this process has central importance in agriculture. In addition, timing of the switch from vegetative to reproductive development in the shoot determines time to maturity of fruit (harvest) and represents one of the most basic elements in adaptation of crop plants to specific growing seasons. Plants with late initiation of flowers mature too late for short growing seasons. Plant architecture or growth habit (vine vs. bush) is another agriculturally important trait that reflects activity or dormancy of the side shoots. In many cases this is controlled by single genes, which when cloned, should allow conversion between bush and vine type growth to suit current agricultural needs.

In the past, growth habit and flowering characteristics of crop plants were manipulated in conventional breeding programs by crossing existing strains and selecting superior individuals from the progeny in field trials. This approach has been successful, but frequently 10-15 years is required before new strains become available for agricultural production.

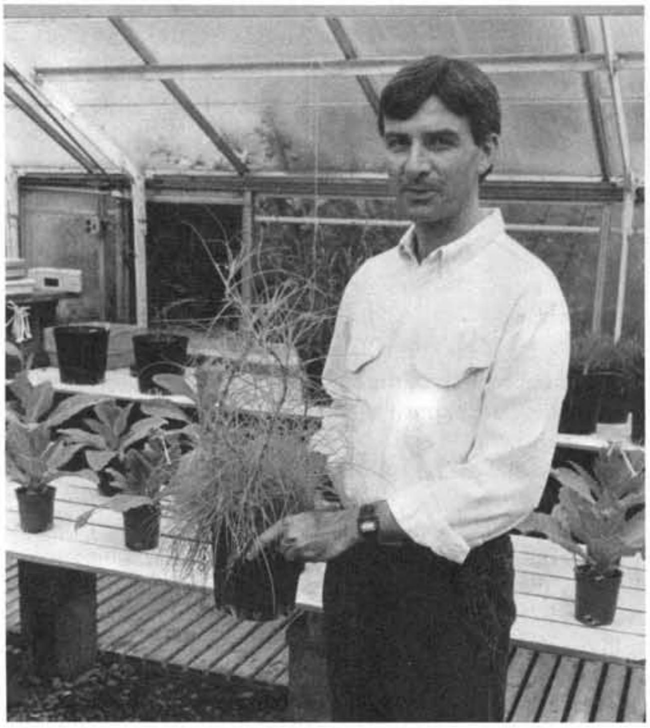

Figure 1. Neil A. McHale with a mutant plant that has no leaf blades.

With the advent of recombinant DNA technology in plants, procedures are being developed that will allow insertion of new genes into existing crop cultivars. This should allow genetic improvement of accepted strains, shortening the time required for testing and acceptance. Consequently, much attention is now focused on identification and cloning of genes controlling agriculturally important traits.

Since procedures for cloning genes in most crop plants are still poorly developed, the current focus in agricultural research is to identify important genes in model plant systems like tobacco and *Arabidopsis* (a small weed in the crucifer family related to cabbage and broccoli) where procedures for molecular cloning have been established. A major benefit in this approach is that genes cloned in model systems frequently provide a direct avenue to cloning the corresponding gene in virtually any other plant. Thus, gene cloning in model systems is expected to greatly accelerate genetic engineering in a broad range of crop plants.

Our current focus is to identify and clone genes that control leaf development. In the early stages of a plant's life cycle, growth is accomplished principally by initiation of new leaves by the shoot apex. The developing leaves have vital importance to the plant because they contain the main photosynthetic tissue supporting plant growth. The photosynthetic cells are located in the leaf blade and arranged in overlapping layers, each of which performs a highly specialized function. The outer layers (epidermis) provide a protective barrier preventing desiccation and have pores allowing gas exchange. Just under the upper epidermis lies the palisade layer which carries out the essential reactions of photosynthesis. The two cell layers of the middle mesophyll differentiate into the secondary vascular system, and the underlying spongy mesophyll is designed for gas diffusion to promote CO_2 uptake and O_2 evolution.

The mechanisms controlling leaf initiation and differentiation of specialized cell layers for light interception and gas exchange remain unknown. This process not only generates the primary photosynthetic cells in the plant, but also establishes the tissue involved in perception of daylength and transmission of developmental signals for stem elongation and flowering. Identifying the genes that control leaf development will establish a foundation for manipulating a variety of agriculturally important traits such as growth habit, early maturity and overall productivity.

Although genes can now be identified by a variety of molecular procedures, the most straightforward approach is to generate a mutation that enhances or blocks a specific function. In practice this is accomplished by generating a broad spectrum of random mutations and then selecting the ones that appear to have hit a gene of interest. Unfortunately these mutations are sometimes lethal to the plant at an early stage of growth, and cannot be recovered for study. In many cases, however, plants carrying a mutation are viable and will display a distinct defect that provides a clue as to the function of the disrupted gene.

To identify genes affecting leaf development, we generated random mutations in tobacco seedlings with ethylmethanesulfonate (EMS) and screened a population of 40,000 individual plants for evidence of defects in the initiation and subsequent development of leaves. We have recovered mutant plants with a wide spectrum of leaf defects. The

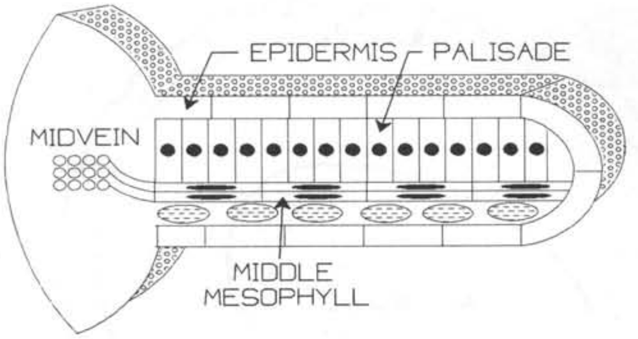

Figure 2. Specialized cell layers in the leaf blade.

most striking was one where development of the leaf blade was completely blocked (lam-*1*).

To learn more about the function of the mutated gene during blade initiation, we examined histological sections of the shoot apex of mutant plants and wild-type counterparts to pinpoint the error. Mutant leaf primordia show active cell division in the two surface cell layers at the normal site of blade initiation, but cell division is defective in the underlying cell layer that normally generates the middle layers of the blade mesophyll. This provides striking evidence that cell layers operate independently during blade formation. Coordination of separate division programs in the surface and underlying cell layers of the leaf is apparently a crucial element in development of the blade. Mutations like *lam-1* provide unique opportunities to determine how patterns of cell division are controlled in the individual cell layers of the blade. Our current objective is to determine the role of the normal gene in formation of photosynthetic cell layers in the leaf.

Ultimately our ability to manipulate plant development will rest on cloning and molecular analysis of the master genes. Model plants like *Arabidopsis* will play a vital role in this endeavor. This plant has an extraordinarily small amount of total DNA among higher plants, and all of its chromosomes are now mapped with genetic markers. This will greatly accelerate the process of gene cloning by pinpointing the physical location of genes on their chromosomes. Accordingly, we have initiated a genetic analysis of leaf development in *Arabidopsis* as an avenue to molecular analysis of the genes involved. This approach has already led to identification and cloning of a variety of genes controlling flower formation by other investigators. Cloning of genes for leaf development will accelerate our progress toward the ultimate goal of engineering the growth and development of agricultural plants.

Controlling yellowjackets and wasps at the Connecticut Tennis Center

By Theodore G. Andreadis and Kenneth A. Welch

Yellowjackets (*Vespula* spp.) and paper wasps (*Polistes* spp.) are valuable scavengers and predators of many insect pests. However, they can become serious pests of man when large numbers forage for food and soft drinks at picnics, fairs, and sporting events. In 1991, they were especially troublesome at the Connecticut Tennis Center during the Volvo International Tennis Tournament that was held in New Haven during August. Twenty-one individuals were treated for "bee stings" and numerous complaints were registered by patrons as menacing numbers of wasps clustered around food and beverage concessions, garbage cans and open-air eating facilities throughout the week-long tournament. The severity of problems with wasps was also apparent in the 239 inquiries on yellowjackets and wasps that were received by the Experiment Station during July and August.

In anticipation of another infestation at the 1992 tournament, the Tennis Foundation of Connecticut, which owns and operates the facility, requested our assistance in controlling wasps.

The primary objective of our wasp management program was to reduce incipient wasp populations to innocuous levels and thereby minimize human encounters and subsequent stings.

Because of the numerous open-air concessions, we felt it would be difficult to modify the facilities to reduce access of wasps to food in the vicinity of human activity. Therefore, we attempted to locate and destroy all nest sites within the spacious complex prior to the start of the tournament. Two methods were used to locate nests and assess wasp activity: traps and thorough inspections. Two types of commercial traps were evaluated: the Seabright Yellow Jacket Inn and the Trappit Yellow Dome (see cover photo). The traps were baited with molasses mixed with a commercial "flying insect attractant" (AgriSence, Fresno, CA) that was composed of a fruit juice residue mixture of sugar and natural fermentation products. Both traps were hung side by side, 3 to 5 feet above the ground, at 24 locations within the facility (Fig. 1). Trapping began the first week of June and continued until the tournament started.

The Yellow Dome Traps were helpful in demonstrating worker activity and showing the general location of one yellowjacket nest. One yellowjacket, *Vespula maculifrons*, was trapped in June, 8 in July, and 32 in August. Sixty-three percent of the yellowjackets were found in traps located within 500 feet of a nest. No yellowjackets or paper wasps were trapped in the Yellow Jacket Inns.

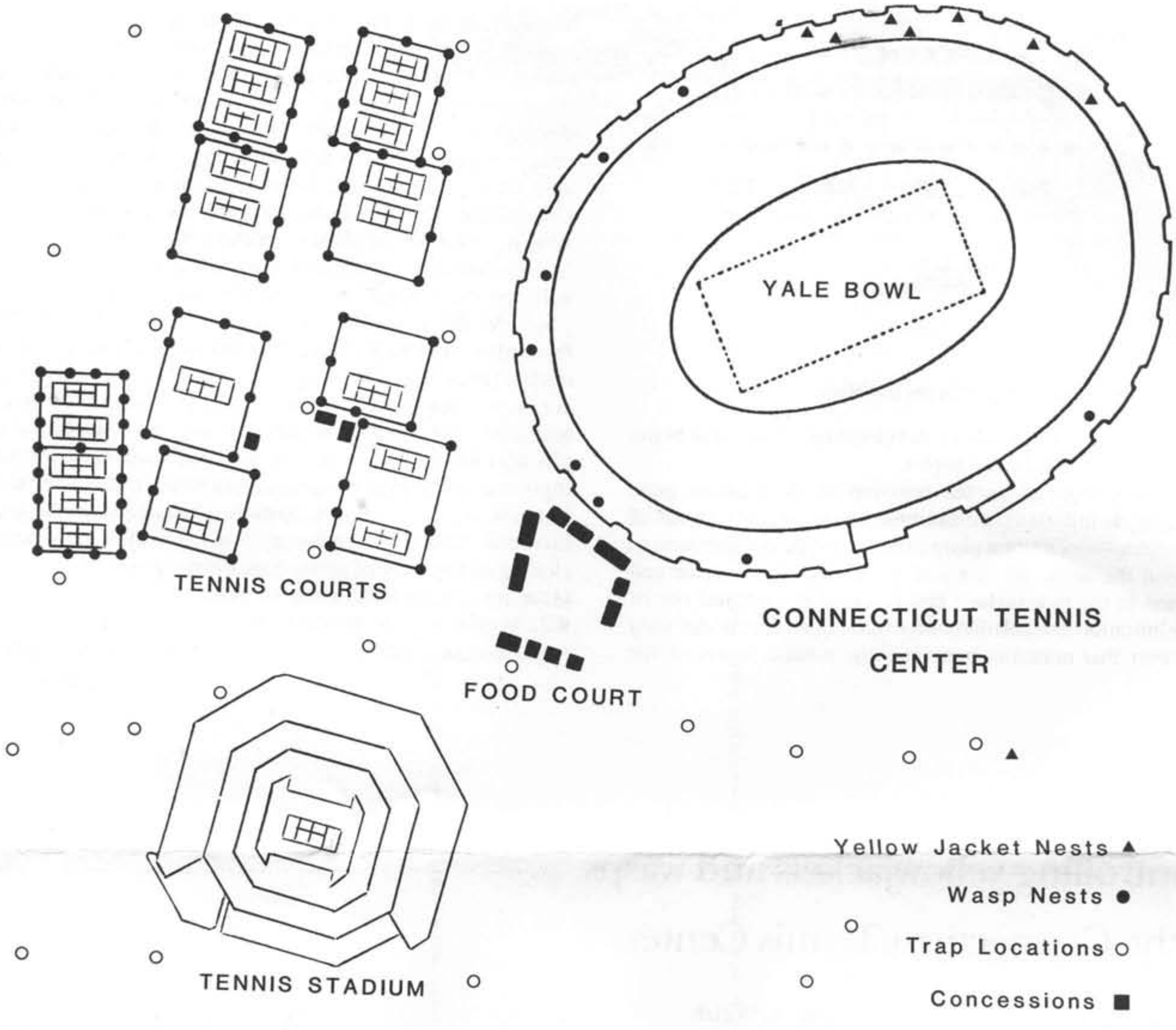

Figure 1. Layout of the Connecticut Tennis Center showing locations where yellowjackets and *Polistes* sp. were found.

Thorough inspection during the first week of August proved to be the most effective method for uncovering individual nests. While surveying the outer grassy rim of the nearby Yale Bowl football stadium, we found seven subterranean yellowjacket nests (approx. 100-200 cells in size), and eight nests (approx. 20-30 cells) of a *Polistes* sp. wasp apparently new to Connecticut (Fig. 1). This *Polistes* sp. wasp was found nesting above ground but hidden under clumps of grass. It has black and yellow markings similar to those of a yellowjacket. Its exact identity and pest potential is not yet known. All nests were individually treated with aerosol insecticide containing chlorpyrifos (Dursban) (0.25%) and allethrin (0.05%) and then dug up or physically destroyed.

Further surveillance on the grounds of the Tennis Center during the first week of August revealed extensive nesting of this same *Polistes* sp. wasp within the small openings atop almost all of the vertical metal fence posts surrounding the tennis courts.

A total of 109 nests were discovered and treated individually with insecticide. Follow-up inspections were made after 24 hours. Retreatment was seldom necessary.

The yellowjacket and wasp management program was highly effective. Few wasps were seen on the grounds of the Tennis Center following treatment and neither wasps nor yellowjackets were a nuisance to the public during the course of the week-long tournament. No pestiferous wasps or yellowjackets were likewise found congregating around the food concessions as in 1991, and we did not receive a single complaint from any of the many food vendors when making our daily inquiries and inspections. Unfortunately, four people were treated for "bee stings" at the medical tent, but these were significantly fewer than the 21 recorded stings in 1991.

We believe the key to the success of the program was the elimination of individual nests, especially those within the fence posts. We cannot be sure whether yellowjackets or the *Polistes* sp. wasp or whether a combination of both were responsible for the problem in 1991. However, the close proximity of the *Polistes* sp. nest sites to the Food Court and other concessions could certainly help explain why wasps were such a problem in 1991.

Ornamental yew shrubs contain significant quantities of taxol

By Mary Jane Incorvia Mattina

Taxol has been described as the most promising anti-cancer drug to be studied in clinical trials in the past decade. However, to investigate the full potential of taxol, sufficient quantities are needed to treat 12,000 patients with a variety of cancers. Unfortunately, the demand for taxol far exceeds the supply.

Taxol was identified in the 1960s in extracts from the Pacific yew tree. The bark of this tree, known scientifically as *Taxus brevifolia*, is the only Food and Drug Administration-approved source of the compound. The Pacific yew, however, is a slow growing tree which is part of the natural habitat of an endangered species, the northern spotted owl. The taxol content of the bark is typically 400 parts-per-million (ppm) or less, and the entire tree must be cut to extract the compound. For these reasons it is desirable to identify alternative sources. At The Connecticut Agricultural Experiment Station, we have been measuring taxol in a potential supply, namely the large number of cultivars of ornamental yews grown commercially in the Northeast. The needles from these other species of the genus *Taxus* are a renewable resource from relatively rapidly growing plants.

The first step was to develop a method for extracting the drug from the needles, removing potentially interfering components and measuring the amount of the drug. The method we use is a modification of a published technique. We remove needles from twigs, grind them in a laboratory blender, and extract them for several hours using methanol. The resulting solution is passed through filter paper, evaporated down to small volume, and cleaned up using a procedure called solid phase extraction. The procedure uses an SPE cartridge, which is a syringe barrel filled with a specially treated powder. A small portion of the extract is transferred to the SPE cartridge. The organic compounds in the extract generally adhere to the powder rather than remain in solution. As the liquid passes through the cartridge, taxol and other extracts remain on the powder. These compounds are then separated from the powder using successively larger amounts of methanol in water. Taxol is separated in the 80% methanol fraction. The impurities remaining in the fraction are then separated from taxol using high performance liquid chromatography. The amount of taxol is measured by UV diode array detection. Figure 2 shows an overlay of the liquid chromatogram using UV detection from a taxol standard (dotted line) and the 80% methanol fraction (solid line) of needles from the cultivar, *Taxus x media* 'Tauntonii'.

Figure 1. Mary Jane Incorvia Mattina (left) and Anthony Paiva (right) examining rooted cuttings of *Taxus x media* 'Nigra'.

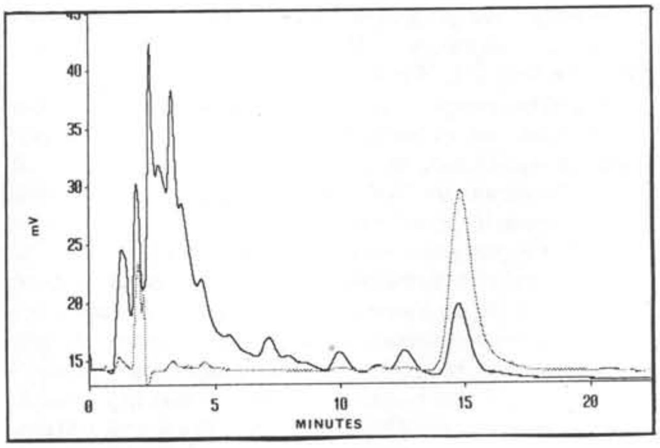

Figure 2. A liquid chromatographic trace of authentic taxol (dotted line) and extract from *Taxus* needles (solid line).

Once we had developed a reliable analytical method, we began our field studies. Arrangements were made with four major *Taxus* growers, The Robert Baker Nurseries in West Suffield, Gardner's Nursery in Rocky Hill, Imperial Nurseries in Granby, and the Rhode Island Nurseries in Newport, RI, to sample all cultivars of field grown shrubs available at each nursery. Three species of *Taxus* were available for sampling, namely, *baccata* (the English yew), *cuspidata* (the Japanese yew), and the hybrid of these, *x media*. Included was one cultivar of the *baccata* species, four cultivars of the *cuspidata* species, and eight cultivars of the *x media* species. In March 1992 we collected samples of twigs with needles from the preceding season's growth ("feathers"). We sampled six to twenty shrubs of each cultivar at random from each nursery. The plant material from each cultivar at a particular nursery was pooled. The material was then processed and the taxol content of the samples determined. The results of these analyses are summarized in Figure 3. This is a graph of the taxol content of the needles on a dry weight basis. Also included are values labeled "Valley" from shrubs located at the Valley Laboratory of The Connecticut Agricultural Experiment Station in Windsor.

The data prompt us to draw several preliminary conclusions. First, one of the cultivars, *Taxus x media* 'Nigra', contains significantly more taxol than any of the other cultivars. At one site the 'Nigra' needle sample contained more than 600 ppm taxol and the 'Nigra' needle sample from the second site contained more than 800 ppm taxol. As the average taxol level in the bark of the Pacific yew is 400 ppm, needles from *Taxus x media* 'Nigra' represent a viable alternative to *T. brevifolia* bark as a taxol source. Second, the site-to-site variation in taxol content of a particular cultivar is relatively small. For example, the taxol content in *T. x media* 'Brownii' varies from a high of 290 ppm to a low of 190 ppm across five sites. Finally, we also observed that there is no apparent correlation between species and taxol content. Thus, 'Sieboldii' with a relatively low and 'Greenwave' with a relatively high taxol level are both members of the *cuspidata* species.

The field study will continue in 1993 with attention focussed on specific 'Nigra' shrubs at two nurseries. We are collecting needles from each shrub monthly to determine if taxol content varies with the season. This will also give us the opportunity to determine the variation in taxol level within a cultivar population.

We are also growing rooted cuttings of certain cultivars in our greenhouses under controlled conditions. We will attempt to determine what effect various treatments have on the taxol content. These include quantity of daylight, water, and nutrients.

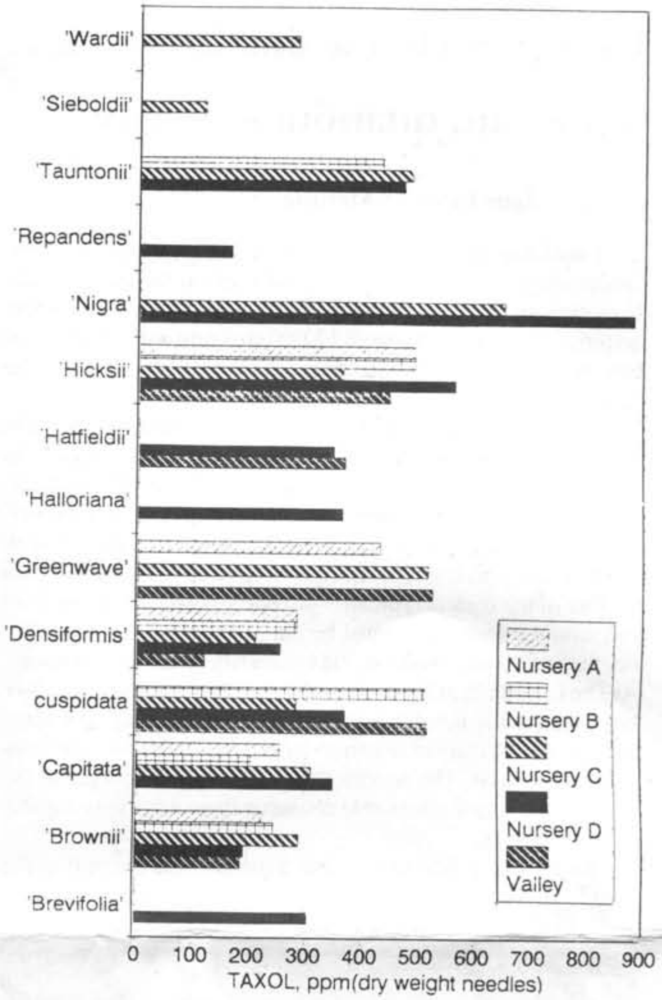

Figure 3. Taxol concentration in needles of ornamental yews.

The ultimate goal of both the field and greenhouse studies is the maximization of taxol yield from field-grown *Taxus*.

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