Marijuana Botany

An Advanced Study: The Propagation and Breeding of Distinctive Cannabis

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Introduction

Cannabis, commonly known in the United States as marijuana, is a wondrous plant an ancient plant and an ally of humanity for over ten thousand years. The profound impact Cannabis has had on the development and spread of civilization and conversely, the profound effects we've had on the plant's evolution are just now being discovered.

Cannabis was one of the earliest and most important plants placed under cultivation by prehistoric Asian peoples. Virtually every part of the plant is usable. From the stem comes hemp, a very long, strong fiber used to make rope, cloth, and paper renowned for durability. The dried leaves and flowers become the euphoriant, marijuana, and along with the root, are also used for numerous medicines. The seeds were a staple food in ancient China, one of their major "grains." Cannabis seeds are somewhat unpalatable and are now cultivated mainly for oil or for animal feed. The oil is similar to linseed and is used for paint and varnish making, fuel, and lubrication.

Cultivated Cannabis quickly spread westward from its native Asia and by Roman times hemp was grown in almost every European country. In Africa, marijuana was the preferred product, smoked both ritually and for pleasure. When the first colonists came to America they, quite naturally, brought hemp seed with them for rope and homespun cloth. Hemp fiber for ships' rigging was so important to the English navy that colonists were paid bounties to grow hemp and in some states, penalties were imposed on those who didn't. Prior to the Civil War, the hemp industry was second only to cotton in the South.

Today, Cannabis grows around the world and is, in fact, considered the most widely distributed of all cultivated plants, a testimony to the plant's tenacity and adaptable nature as well as to its usefulness and economic value. Unlike many plants, Cannabis never lost the ability to flourish without human help despite, perhaps, six millennia of cultivation.

Whenever ecological circumstances permit, the plants readily "escape" cultivation by becoming weedy and establishing "wild" populations. Weedy Cannabis, descended from the bygone hemp industry, grows in all but the more arid areas of the United States. Unfortunately, these weeds usually make a very poor grade marijuana.

Such an adaptable plant, brought to a wide range of environments, and cultivated and bred for a multitude of products, understandably evolved a great number of

distinctive strains or varieties, each one uniquely suited to local needs and growing conditions. Many of these varieties may be lost through extinction and hybridization unless a concerted effort is made to preserve them. This book provides the basis for such an undertaking.
There are likely more varieties of marijuana being grown or held as seeds in this country than any other. While traditional marijuana growers in Asia and Africa, typically, grow the same, single variety their forebears grew, American growers seek and embrace varieties from all parts of the world. Very potent, early flowering varieties are especially prized because they can complete maturation even in the northernmost states. The Cannabis stock in the United Nations seed bank is at best, depleted and in disarray. American growers are in the best position to prevent further loss of valuable varieties by saving, cataloguing, and propagating their seeds.

Marijuana Botany - the Propagation and Breeding of Distinctive Cannabis is an important and most welcome book. Its main thrust is the presentation of the scientific and horticultural principles, along with their practical applications, necessary for the breeding and propagation of Cannabis and in particular, marijuana. This book will appeal not only to the professional researcher, but to the marijuana enthusiast or anyone with an eye to the future of Cannabis products.

To marijuana growers who wish to improve or upgrade their varieties, the book is an invaluable reference. Basic theories and practices for breeding pure stock or hybrids, cloning, grafting, or breeding to improve quali ties such as potency or yield, are covered in a clear, easy-to-follow text which is liberally complemented with drawings, charts, and graphs by the author. Rob Clarke's drawings reflect his love of Cannabis. They sensitively capture the plant's elegance and everchanging beauty while being always informative and accurately rendered.

The reader not familiar with botanical terms need not be intimidated by a quick glance at the text. All terms are defined when they are introduced and there is also a glossary with definitions geared to usage. Anyone familiar with the plant will easily adopt the botanical terms.

Years from now, many a marijuana smoker may unknowingly be indebted to this book for the exotic varieties that will be preserved and new ones that will be developed. Growers will especially appreciate the expert information on marijuana propagation and breeding so attractively and clearly presented.

Mel Frank
author, Marijuana Growers' Guide

Preface

Turn again our captivity, 0 Lord,
as the streams in the dry land.
They that sow in tears shall reap in joy.
He that goeth forth and weepeth,
bearing precious seed,
shall doubtless come again with rejoicing, bringing his sheaves with him.
- Psalms 126: 4-6

Cannabis is one of the world's oldest cultivated plants. Currently, however, Cannabis cultivation and use is illegal or legally restricted around the globe. Despite constant official control, Cannabis cultivation and use has spread to every continent and nearly every nation. Cultivated and wild Cannabis flourishes in temperate and tropical climates worldwide. Three hundred million users form a strong undercurrent beneath the flowing tide of eradication. To judge by increasing official awareness of the economic potentials of Cannabis, legalization seems inevitable although slow. Yet as Cannabis faces eventual legalization it is threatened by extinction. Government sanctioned and supported spraying with herbicides and other forms of eradication have chased ancient Cannabis strains from their native homes.

Cannabis has great potential for many commercial uses. According to a recent survey of available research by Turner, Elsohly and Boeren (1980) of the Research Institute of Pharmaceutical Sciences at the University of Mississippi, Cannabis contains 421 known compounds, and new ones are constantly being discovered and reported. Without further understanding of the potentials of Cannabis as a source of fiber, fuel, food, industrial chemicals and medicine it seems thoughtless to support eradication campaigns.

World politics also threaten Cannabis. Rural Cannabis farming cultures of the Middle East, Southeast Asia, Cen tral America and Mrica face political unrest and open aggression. Cannabis seeds cannot be stored forever. If they are not planted and reproduced each year a strain could be lost. Whales, big cats, and redwoods are all protected in preserves established by national and international laws. Plans must also be implemented to protect Cannabis cultures and rare strains from certain extinction.

Agribusiness is excited at the prospect of supplying America's 20 million Cannabis users with domestically grown commercial marijuana. As a result, development of uniform patented hybrid strains by multinational agricultural firms is inevitable. The morality of plant patent laws has been challenged for years. For humans to recombine and then patent the genetic material of another living organism, especially at the expense of the original organism, certainly offends the moral sense of many concerned citizens. Does the slight recombination of a plant's genetic material by a breeder give him the right to own that organism and its offspring? Despite public resistance voiced by conservation groups, the Plant Variety Protection Act of 1970 was passed and currently allows the patenting of 224 vegetable crops. New amendments could grant patent holders exclusive rights for 18 years to distribute, import, export and use for breeding purposes their newly developed strains. Similar conventions worldwide could further threaten genetic resources. Should patented varieties of Cannabis become reality it might be illegal to grow any strain other than a patented variety, especially for food or medicinal uses. Limitations could also be imposed such that only low THC strains would be patentable. This could lead to restrictions on small scale growing of Cannabis; commercial growers could not take the chance of stray pollinations from private plots harming a valuable seed crop. Proponents of plant patenting claim that patents will encourage the development of new varieties. In fact, patent laws encourage the spread of uniform strains devoid of the genetic diversity which allows improvements. Patent laws have also fostered intense competition between breeders and the suppression of research results which if made public could speed crop improvement. A handful of large corporations hold the vast majority of plant patents. These conditions will make it impossible for cultivators of native strains to compete with agribusiness and could lead to the further extinction of native strains now surviving on small farms in North America and Europe. Plant improvement in itself presents no threat to genetic reserves. However, the support and spread of improved strains by large corporations could prove disastrous.

Like most major crops, Cannabis originated outside North America in still primitive areas of the world. Thousands of years ago humans began to gather seeds from wild Cannabis and grow them in fields alongside the first cultivated food crops. Seeds from the best plants were saved for planting the following season. Cannabis was spread by nomadic tribes and by trade between cultures until it now appears in both cultivated and escaped forms in many nations. The pressures of human and natural selection have resulted in many distinct strains adapted to unique niches within the ecosystem. Thus, individual Cannabis strains possess unique gene pools containing great potential diversity. In this diversity lies the strength of genetic inheritance. From diverse gene pools breeders extract the desirable traits incorporated into new varieties. Nature also calls on the gene pool to ensure that a strain will survive. As climate changes and stronger pests and diseases appear, Cannabis evolves new adaptations and defenses.

Modern agriculture is already striving to change this natural system. When Cannabis is legalized, the breeding and marketing of improved varieties for commercial agriculture is certain. Most of the areas suitable for commercial Cannabis cultivation already harbor their own native strains. Improved strains with an adaptive edge will follow in the wake of commercial agriculture and replace rare native strains in foreign fields. Native strains will hybridize with introduced strains through windborne pollen dispersal and some genes will be squeezed from the gene pool.

Herein lies extreme danger! Since each strain of Cannabis is genetically unique and contains at least a few genes not found in other strains, if a strain becomes extinct the unique genes are lost forever. Should genetic weaknesses arise from excessive inbreeding of commercial strains, new varieties might not be resistant to a previously unrecognized environmental threat. A disease could spread rapidly and wipe out entire fields simultaneously. Widespread crop failure would result in great financial loss to the farmer and possible extinction of entire strains.

In 1970, to the horror of American farmers and plant breeders, Southern corn leafblight (Helm in thosporium maydis) spread quickly and unexpectedly throughout corn crops and caught farmers off guard with no defense. H. maydis is a fungus which causes minor rot and damage in corn and had previously had no economic impact. However, in 1969 a virulent mutant strain of the fungus appeared in Illinois, and by the end of the following season its windborne spores had spread and blighted crops from the Great Lakes to the Gulf of Mexico. Approximately 15% of America's corn crop was destroyed. In some states over half the crop was lost.

Fortunately the only fields badly infected were those containing strains descended from parents of what corn breeders called "the Texas strain." Plants descended from parents of previously developed strains were only slightly infected. The discovery and spread of the Texas strain had revolutionized the corn industry. Since pollen from this strain is sterile, female plants do not have to be detasseled by hand or machine, saving farmers millions of dollars annually. Unknown to corn breeders, hidden in this improved strain was an extreme vulnerability to the mutant leafblight fungus.

Total disaster was avoided by the around the clock efforts of plant breeders to develop a commercial strain from other than Texas plants. It still took three years to develop and reproduce enough resistant seed to supply all who needed it. We are also fortunate that corn breeders could rise to the challenge and had maintained seed reserves for breeding. If patented hybrid strains of Cannabis are produced and gain popularity, the same situation could arise. Many pathogens are known to infect Cannabis and any one of them has the potential to reach epidemic proportions in a genetically uniform crop. We can not and should not stop plant improvement programs and the use of hybrid strains. However, we should provide a reserve of genetic material in case it is required in the future.

Breeders can only combat future problems by relying on primitive gene pools contained in native strains. If native gene pools have been squeezed out by competition from patented commercial hybrids than the breeder is helpless. The forces of mutation and natural selection take thousands of years to modify gene pools, while a Cannabis blight could spread like wildfire.

As Cannabis conservationists, we must fight the further amendment of plant patent laws to include Cannabis, and initiate programs immediately to collect, catalogue, and propagate vanishing strains. Cannabis preserves are needed where each strain can be freely cultivated in areas resembling native habitats. This will help reduce the selective pressure of an introduced environment, and preserve the genetic integrity of each strain. Presently such a program is far from becoming a reality and rare strains are vanishing faster than they can be saved. Only a handful of dedicated researchers, cultivators, and conservationists are concerned with the genetic fate of Cannabis. It is tragic that a plant with such promise should be caught up in an age when extinction at the hands of humans is commonplace. Responsibility is left with the few who will have the sensitivity to end genocide and the foresight to preserve Cannabis for future generations.

Marijuana Botany presents the scientific knowledge and propagation techniques used to preserve and multiply vanishing Cannabis strains. Also included is information concerning Cannabis genetics and breeding used to begin plant improvement programs. It is up to the individual to use this information thoughtfully and responsibly.

Chapter 1 - Sinsemilla Life Cycle of Cannabis

Cannabis is a tall, erect, annual herb. Provided with an open sunny environment, light well-drained composted soil, and ample irrigation, Cannabis can grow to a height of 6 meters (about 20 feet) in a 4-6 month growing season. Exposed river banks, meadows, and agricultural lands are ideal habitats for Cannabis since all offer good sunlight. In this example an imported seed from Thailand is grown without pruning and becomes a large female plant. A cross with a cutting from a male plant of Mexican origin results in hybrid seed which is stored for later planting. This example is representative of the outdoor growth of Cannabis in temperate climates.

Seeds are planted in the spring and usually germinate in 3 to 7 days. The seedling emerges from the ground by the straightening of the hypocotyl (embryonic stem). The cotyledons (seed leaves) are slightly unequal in size, narrowed to the base and rounded or blunt to the tip. The hypocotyl ranges from 1 to 10 centimeters (1A to 3 inches) in length. About 10 centimeters or less above the cotyledons, the first true leaves arise, a pair of oppositely oriented single leaflets each with a distinct petiole (leaf stem) rotated one-quarter turn from the cotyledons. Subsequent pairs of leaves arise in opposite formation and a variously shaped leaf sequence develops with the second pair of leaves having 3 leaflets, the third 5 and so on up to 11 leaflets. Occasionally the first pair of leaves will have 3 leaflets each rather than 1 and the second pair, 5 leaflets each.

If a plant is not crowded, limbs will grow from small buds (located at the intersection of petioles) along the main stem. Each sinsemilla (seedless drug Cannabis) plant is provided with plenty of room to grow long axial limbs and extensive fine roots to increase floral production. Under favorable conditions Cannabis grows up to 7 centimeters (21A inches) a day in height during the long days of summer.

Cannabis shows a dual response to daylength; during the first two to three months of growth it responds to increasing daylength with more vigorous growth, but in the same season the plant requires shorter days to flower and complete its life cycle.

LIFE CYCLE OF CANNABIS I Juvenile Stage

Cannabis flowers when exposed to a critical daylength which varies with the strain. Critical daylength applies only to plants which fail to flower under continuous illumination, since those which flower under continuous illumination have no critical daylength. Most strains have an absolute requirement of inductive photoperiods (short days or long nights) to induce fertile flowering and less than this will result in the formation of undifferentiated primordia (unformed flowers) only.

The time taken to form primordia varies with the length of the inductive photoperiod. Given 10 hours per day of light a strain may only take 10 days to flower, whereas if given 16 hours per day it may take up to 90 days. Inductive photoperiods of less than 8 hours per day do not seem to accelerate primordia formation. Dark (night) cycles must be uninterrupted to induce flowering (see appendix).

Cannabis is a dioecious plant, which means that the male and female flowers develop on separate plants, although monoecious examples with both sexes on one plant are found. The development of branches containing flowering organs varies greatly between males and females: the male flowers hang in long, loose, multi-branched, clustered limbs up to 30 centimeters (12 inches) long, while the female flowers are tightly crowded between small leaves.

Note: Female Cannabis flowers and plants will be referred to as pistillate and male flowers and plants will be referred to as staminate in the remainder of this text. This convention is more accurate and makes examples of complex aberrant sexuality easier to understand.

The first sign of flowering in Cannabis is the appearance of undifferentiated flower primordia along the main stem at the nodes (intersections) of the petiole, behind the stipule (leaf spur). In the prefloral phase, the sexes of Cannabis are indistinguishable except for general trends in shape.

When the primordia first appear they are undifferentiated sexually, but soon the males can be identified by their curved claw shape, soon followed by the differentiation of round pointed flower buds having five radial segments. The females are recognized by the enlargement of a symmetrical tubular calyx (floral sheath). They are easier to recognize at a young age than male primordia. The first female calyxes tend to lack paired pistils (pollen-catching appendages) though initial male flowers often mature and shed viable pollen. In some individuals, especially hybrids, small non-flowering limbs will form at the nodes and are often confused with male primordia.

Cultivators wait until actual flowers form to positively determine the sex of Cannabis

The female plants tend to be shorter and have more branches than the male. Female plants are leafy to the top with many leaves surrounding the flowers, while male plants have fewer leaves near the top with few if any leaves along the extended flowering limbs.

*The term pistil has developed a special meaning with respect to Cannabis which differs slightly from the precise botanical definition. This has come about mainly from the large number of cultivators who have casual knowledge of plant anatomy but an intense interest in the reproduction of Cannabis. The precise definition of pistil refers to the combination of ovary, style and stigma. In the more informal usage, pistil refers to the fused style and stigma. The informal sense is used throughout the book since it has become common practice among Cannabis cultivators.

The female flowers appear as two long white, yellow, or pink pistils protruding from the fold of a very thin membranous calyx. The calyx is covered with resin exuding glandular trichomes (hairs). Pistillate flowers are borne in pairs at the nodes one on each side of the petiole behind the stipule of bracts (reduced leaves) which conceal the flowers. The calyx measures 2 to 6 millimeters in length and is closely applied to, and completely contains, the ovary.

In male flowers, five petals (approximately 5 millimeters, or 3/16 inch, long) make up the calyx and may be yellow, white, or green in color. They hang down, and five stamens (approximately 5 millimeters long) emerge, consisting of slender anthers (pollen sacs), splitting upwards from the tip and suspended on thin filaments. The exterior surface of the staminate calyx is covered with non-glandular trichomes. The pollen grains are nearly spherical slightly yellow, and 25 to 30 microns (p) in diameter. The surface is smooth and exhibits 2 to 4 germ pores.

Before the start of flowering, the phyllotaxy (leaf arrangement) reverses and the number of leaflets per leaf decreases until a small single leaflet appears below each pair of calyxes. The phyllotaxy also changes from decussate (opposite) to alternate (staggered) and usually remains alternate throughout the floral stages regardless of sexual type.

The differences in flowering patterns of male and female plants are expressed in many ways. Soon after dehiscence (pollen shedding) the staminate plant dies, while the pistillate plant may mature up to five months after viable flowers are formed if little or no fertilization occurs. Compared with pistillate plants, staminate plants show a more rapid increase in height and a more rapid decrease in leaf size to the bracts which accompany the flowers. Staminate plants tend to flower up to one month earlier than pistillate plants; however, pistillate plants often differentiate primordia one to two weeks before staminate plants.

Many factors contribute to determining the sexuality of a flowering Cannabis plant. Under average conditions with a normal inductive photoperiod, Cannabis will bloom and produce approximately equal numbers of pure staminate and pure pistillate plants with a few hermaphrodites (both sexes on the same plant). Under conditions of extreme stress, such as nutrient excess or deficiency, mutilation, and altered light cycles, populations have been shown to depart greatly from the expected one-to-one staminate to pistillate ratio.

Just prior to dehiscence, the pollen nucleus divides to produce a small reproductive cell accompanied by a large vegetative cell, both of which are contained within the mature pollen grain. Germination occurs 15 to 20 minutes after contact with a pistil. As the pollen tube grows the vegetative cell remains in the pollen grain while the generative cell enters the pollen tube and migrates toward the ovule. The generative cell divides into two gametes (sex cells) as it travels the length of the pollen tube.

Pollination of the pistillate flower results in the loss of the paired pistils and a swelling of the tubular calyx where the ovule is enlarging. The staminate plants die after shedding pollen. After approximately 14 to 35 days the seed is matured and drops from the plant, leaving the dry calyx attached to the stem. This completes the normally 4 to 6 month life cycle, which may take as little as 2 months or as long as 10 months. Fresh seeds approach 100% viability, but this decreases with age.

The hard mature seed is partially surrounded by the calyx and is variously patterned in grey, brown, or black. Elongated and slightly compressed, it measures 2 to 6 millimeters (1/16 to 3/16 inch) in length and 2 to 4 millimeters (1/16 to 1/8 inch) in maximum diameter.

Careful closed pollinations of a fewselected limbs yield hundreds of seeds of known parentage, which are removed after they are mature and beginning to fall from the calyxes. The remaining floral clusters are sinsemilla or seedless and continue to mature on the plant. As the unfertilized calyxes swell, the glandular trichomes on the surface grow and secrete aromatic THC-laden resins. The mature, pungent, sticky floral clusters are harvested, dried, and sampled. The preceding simplified life cycle of sinsemilla Cannabis exemplifies the production of valuable seeds without compromising the production of seedless floral clusters.

Chapter 2 - Propagation of Cannabis

"Make the most of the Indian Hemp Seed and sow it every where."

- George Washington

Sexual versus Asexual Propagation

Cannabis can be propagated either sexually or asexually. Seeds are the result of sexual propagation. Because sexual propagation involves the recombination of genetic material from two parents we expect to observe variation among seedlings and offspring with characteristics differing from those of the parents. Vegetative methods of propagation (cloning) such as cuttage, layerage, or division of roots are asexual and allow exact replication of the parental plant without genetic variation. Asexual propagation, in theory, allows strains to be preserved unchanged through many seasons and hundreds of individuals.

When the difference between sexual and asexual propagation is well understood then the proper method can be chosen for each situation. The unique characteristics of a plant result from the combination of genes in chromosomes present in each cell, collectively known as the genotype of that individual. The expression of a genotype, as influenced by the environment, creates a set of visible characteristics that we collectively term the phenotype. The function of propagation is to preserve special genotypes by choosing the proper technique to ensure replication of the desired characteristics.

If two clones from a pistillate Cannabis plant are placed in differing environments, shade and sun for in stance, their genotypes will remain identical. However, the clone grown in the shade will grow tall and slender and mature late, while the clone grown in full sun will remain short and bushy and mature much earlier.

Sexual Propagation

Sexual propagation requires the union of staminate pollen and pistillate ovule, the formation of viable seed, and the creation of individuals with newly

recombinant genotypes. Pollen and ovules are formed by reduction divisions (meiosis) in which the 10 chromosome pairs fail to replicate, so that each of the two daughter-cells contains one-half of the chromosomes from the mother cell. This is known as the haploid (in) condition where in = 10 chromosomes. The diploid condition is restored upon fertilization resulting in diploid (2n) individuals with a haploid set of chromosomes from each parent. Offspring may resemble the staminate, pistillate, both, or neither parent and considerable variation in offspring is to be expected. Traits may be controlled by a single gene or a combination of genes, resulting in further potential diversity.

The terms homozygous and heterozygous are useful in describing the genotype of a particular plant. If the genes controlling a trait are the same on one chromosome as those on the opposite member of the chromosome pair (homologous chromosomes), the plant is homozygous and will "breed true" for that trait if self-pollinated or crossed with an individual of identical genotype for that trait. The traits possessed by the homozygous parent will be transmitted to the offspring, which will resemble each other and the parent. If the genes on one chromosome differ from the genes on its homologous chromosome then the plant is termed heterozygous; the resultant offspring may not possess the parental traits and will most probably differ from each other. Imported Cannabis strains usually exhibit great seedling diversity for most traits and many types will be discovered.

To minimize variation in seedlings and ensure preservation of desirable parental traits in offspring, certain careful procedures are followed as illustrated in Chapter III. The actual mechanisms of sexual propagation and seed production will be thoroughly explained here.

The Life Cycle and Sinsemilla Cultivation

A wild Cannabis plant grows from seed to a seedling, to a prefloral juvenile, to either pollen- or seed-bearing adult, following the usual pattern of development and sexual reproduction. Fiber and drug production both interfere with the natural cycle and block the pathways of inheritance. Fiber crops are usually harvested in the juvenile or prefloral stage, before viable seed is produced, while sinsemilla or seedless marijuana cultivation eliminates pollination and subsequent seed production. In the case of cultivated Cannabis crops, special techniques must be used to produce viable seed for the following year without jeopardizing the quality of the final product.

Modern fiber or hemp farmers use commercially produced high fiber content strains of even maturation. Monoecious strains are often used because they mature more evenly than dioecious strains. The hemp breeder sets up test plots where phenotypes can be recorded and controlled crosses can be made. A farmer may leave a portion of his crop to develop mature seeds which he collects

for the following year. If a hybrid variety is grown, the offspring will not ail resemble the parent crop and desirable characteristics may be lost.

Growers of seeded marijuana for smoking or hashish production collect vast quantities of seeds that fall from the flowers during harvesting, drying, and processing. A mature pistillate plant can produce tens of thousands of seeds if freely pollinated. Sinsemilla marijuana is grown by removing all the staminate plants from a patch, eliminating every pollen source, and allowing the pistillate plants to produce massive clusters of unfertilized flowers.

Various theories have arisen to explain the unusually potent psychoactive properties of unfertilized Cannabis. In general these theories have as their central theme the extraordinarily long, frustrated struggle of the pistillate plant to reproduce, and many theories are both twisted and romantic. What actually happens when a pistillate plant remains unfertilized for its entire life and how this ultimately affects the cannabinoid (class of molecules found only in Cannabis) and terpene (a class of aromatic organic compounds) levels remains a mystery. It is assumed, how ever, that seeding cuts the life of the plant short and THC (tetrahydrocannabinol the major psychoactive compound in Cannabis) does not have enough time to accumulate. Hormonal changes associated with seeding definitely affect all metabolic processes within the plant including cannabinoid biosynthesis. The exact nature of these changes is unknown but probably involves imbalance in the enzymatic systems controlling cannabinoid production. Upon fertilization the plant’s energies are channeled into seed production instead of increased resin production. Sinsemilla plants continue to produce new floral clusters until late fail, while seeded plants cease floral production. It is also suspected that capitate-stalked trichome production might cease when the calyx is fertilized. If this is the case, then sinsemilla may be higher in THC because of uninterrupted floral growth, trichome formation and cannabinoid production. What is important with respect to propagation is that once again the farmer has interfered with the life cycle and no naturally fertilized seeds have been produced.

The careful propagator, however, can produce as many seeds of pure types as needed for future research without risk of pollinating the precious crop. Staminate parents exhibiting favorable characteristics are reproductively isolated while pollen is carefully collected and applied to only selected flowers of the pistillate parents.

Many cultivators overlook the staminate plant, considering it useless if not detrimental. But the staminate plant contributes half of the genotype expressed in the offspring. Not only are staminate plants preserved for breeding, but they must be allowed to mature, uninhibited, until their phenotypes can be determined and the most favorable individuals selected. Pollen may also be stored for short periods of time for later breeding.

Biology of Pollination

Pollination is the event of pollen landing on a stigmatic surface such as the pistil, and fertilization is the union of the staminate chromosomes from the pollen with the pistillate chromosomes from the ovule.

Pollination begins with dehiscence (release of pollen) from staminate flowers. Millions of pollen grains float through the air on light breezes, and many land on the stigmatic surfaces of nearby pistillate plants. If the pistil is ripe, the pollen grain will germinate and send out a long pollen tube much as a seed pushes out a root. The tube contains a haploid (in) generative nucleus and grows downward toward the ovule at the base of the pistils. When the pollen tube reaches the ovule, the staminate haploid nucleus fuses with the pistillate haploid nucleus and the diploid condition is restored. Germination of the pollen grain occurs 15 to 20 minutes after contact with the stigmatic surface (pistil); fertilization may take up to two days in cooler temperatures. Soon after fertilization, the pistils wither away as the ovule and surrounding calyx begin to swell. If the plant is properly watered, seed will form and sexual reproduction is complete. It is crucial that no part of the cycle be interrupted or viable seed will not form. If the pollen is subjected to extremes of temperature, humidity, or moisture, it will fail to germinate, the pollen tube will die prior to fertilization, or the embryo will be unable to develop into a mature seed. Techniques for successful pollination have been designed with all these criteria in mind.

Controlled versus Random Pollinations

The seeds with which most cultivators begin represent varied genotypes even when they originate from the same floral cluster of marijuana, and not all of these genotypes will prove favorable. Seeds collected from imported shipments are the result of totally random pollinations among many genotypes. If elimination of pollination was at tempted and only a few seeds appear, the likelihood is very high that these pollinations were caused by a late flowering staminate plant or a hermaphrodite, adversely affecting the genotype of the offspring. Once the offspring of imported strains are in the hands of a competent breeder, selection and replication of favorable phenotypes by controlled breeding may begin. Only one or two individuals out of many may prove acceptable as parents. If the cultivator allows random pollination to occur again, the population not only fails to improve, it may even degenerate through natural and accidental selection of unfavorable traits. We must therefore turn to techniques of controlled pollination by which the breeder attempts to take control and deter mine the genotype of future offspring.

Data Collection

Keeping accurate notes and records is a key to successful plant-breeding. Crosses among ten pure strains (ten staminate and ten pistillate parents) result in ten pure and ninety hybrid crosses. It is an endless and inefficient task to attempt to remember the significance of each little number and colored tag associated with each cross. The well organized breeder will free himself from this mental burden and possible confusion by entering vital data about crosses, phenotypes, and growth conditions in a system with one number corresponding to each member of the population.

The single most important task in the proper collection of data is to establish undeniable credibility. Memory fails, and remembering the steps that might possibly have led to the production of a favorable strain does not constitute the data needed to reproduce that strain. Data is always written down; memory is not a reliable record. A record book contains a numbered page for each plant, and each separate cross is tagged on the pistillate parent and recorded as follows: "seed of pistillate parent X pollen or staminate parent." Also the date of pollination is included and room is left for the date of seed harvest. Samples of the parental plants are saved as voucher specimens for later characterization and analysis.

Pollination Techniques

Controlled hand pollination consists of two basic steps: collecting pollen from the anthers of the staminate parent and applying pollen to the receptive stigmatic surfaces of the pistillate parent. Both steps are carefully con trolled so that no pollen escapes to cause random pollinations. Since Cannabis is a wind-pollinated species, enclosures are employed which isolate the ripe flowers from wind, eliminating pollination, yet allowing enough light penetration and air circulation for the pollen and seeds to develop without suffocating. Paper and very tightly woven cloth seem to be the most suitable materials. Coarse cloth allows pollen to escape and plastic materials tend to collect transpired water and rot the flowers. Light-colored opaque or translucent reflective materials remain cooler in the sun than dark or transparent materials, which either absorb solar heat directly or create a greenhouse effect, heating the flowers inside and killing the pollen. Pollination bags are easily constructed by gluing together vegetable parchment (a strong breathable paper for steaming vegetables) and clear nylon oven bags (for observation windows) with silicon glue. Breathable synthetic fabrics such as Gore-Tex are used with great success. Seed production requires both successful pollination and fertilization, so the conditions inside the enclosures must remain suitable for pollen-tube growth and fertilization. It is most convenient and effective to use the same enclosure to collect pollen and apply it, reducing contamination during pollen transfer. Controlled "free" pollinations may also be made if only one pollen parent is allowed to remain in an isolated area of the field and no pollinations are caused by hermaphrodites or late-maturing

staminate plants. If the selected staminate parent drops pollen when there are only a few primordial flowers on the pistillate seed parent, then only a few seeds will form in the basal flowers and the rest of the flower cluster will be seedless. Early fertilization might also help fix the sex of the pistillate plant, helping to prevent hermaphrodism. Later, hand pollinations can be performed on the same pistillate parent by removing the early seeds from each limb to be re-pollinated, so avoiding confusion. Hermaphrodite or monoecious plants may be isolated from the remainder of the population and allowed to freely self-pollinate if pure-breeding offspring are desired to preserve a selected trait. Selfed hermaphrodites usually give rise to hermaphrodite offspring.

Pollen may be collected in several ways. If the propagator has an isolated area where staminate plants can grow separate from each other to avoid mutual contamination and can be allowed to shed pollen without endangering the remainder of the population, then direct collection may be used. A small vial, glass plate, or mirror is held beneath a recently-opened staminate flower which appears to be releasing pollen, and the pollen is dislodged by tap ping the anthers. Pollen may also be collected by placing whole limbs or clusters of staminate flowers on a piece of paper or glass and allowing them to dry in a cool, still place. Pollen will drop from some of the anthers as they dry, and this may be scraped up and stored for a short time in a cool, dark, dry spot. A simple method is to place the open pollen vial or folded paper in a larger sealable container with a dozen or more fresh, dry soda crackers or a cup of dry white rice. The sealed container is stored in the refrigerator and the dry crackers or rice act as a desiccant, absorbing moisture from the pollen.

Any breeze may interfere with collection and cause contamination with pollen from neighboring plants. Early morning is the best time to collect pollen, as it has not been exposed to the heat of the day. All equipment used for collection, including hands, must be cleaned before continuing to the next pollen source. This ensures protection of each pollen sample from contamination with pollen from different plants.

Staminate flowers will often open several hours before the onset of pollen release. If flowers are collected at this time they can be placed in a covered bottle where they will open and release pollen within two days. A carefully sealed paper cover allows air circulation, facilitates the release of pollen, and prevents mold.

Both of the previously described methods of pollen collection are susceptible to gusts of wind, which may cause contamination problems if the staminate pollen plants grow at all close to the remaining pistillate plants. There fore, a method has been designed so that controlled pollen collection and application can be performed in the same area without the need to move staminate plants from their original location. Besides the advantages of convenience, the pollen parents

mature under the same conditions as the seed parents, thus more accurately expressing their phenotypes.

The first step in collecting pollen is, of course, the selection of a staminate or pollen parent. Healthy individuals with well-developed clusters of flowers are chosen. The appearance of the first staminate primordia or male sex signs often brings a feeling of panic ("stamenoia") to the cultivator of seedless Cannabis, and potential pollen parents are prematurely removed. Staminate primordia need to develop from one to five weeks before the flowers open and pollen is released. During this period the selected pollen plants are carefully watched, daily or hourly if necessary, for developmental rates vary greatly and pollen may be released quite early in some strains. The remaining staminate plants that are unsuitable for breeding are destroyed and the pollen plants specially labeled to avoid confusion and extra work.

As the first flowers begin to swell, they are removed prior to pollen release and destroyed. Tossing them on the ground is ineffective because they may release pollen as they dry. When the staminate plant enters its full floral condition and more ripe flowers appear than can be easily controlled, limbs with the most ripe flowers are chosen. It is usually safest to collect pollen from two limbs for each intended cross, in case one fails to develop. If there are ten prospective seed parents, pollen from twenty limbs on the pollen parent is collected. In this case, the twenty most flowered limb tips are selected and all the remaining flowering clusters on the plant are removed to prevent stray pollinations. Large leaves are left on the remainder of the plant but are removed at the limb tips to minimize condensation of water vapor released inside the enclosure. The portions removed from the pollen parent are saved for later analysis and phenotype characterization.

The pollination enclosures are secured and the plant is checked for any shoots where flowers might develop outside the enclosure. The completely open enclosure is slipped over the limb tip and secured with a tight but stretchable seal such as a rubber band, elastic, or plastic plant tie-tape to ensure a tight seal and prevent crushing of the vascular tissues of the stem. String and wire are avoided. If enclosures are tied to weak limbs they may be supported; the bags will also remain cooler if they are shaded. Hands are always washed before and after handling each pollen sample to prevent accidental pollen transfer and contamination.

Enclosures for collecting and applying pollen and preventing stray pollination are simple in design and construction. Paper bags make convenient enclosures. Long narrow bags such as light-gauge quart-bottle bags, giant popcorn bags or bakery bags provide a convenient shape for covering the limb tip. The thinner the paper used the more air circulation is allowed, and the better the flowers will develop. Very thick paper or plastic bags are never used. Most available bags are made with water soluble glue and may come apart after rain or watering. All

seams are sealed with waterproof tape or silicon glue and the bags should not be handled when wet since they tear easily. Bags of Gore-Tex cloth or vegetable parchment will not tear when wet. Paper bags make labeling easy and each bag is marked in waterproof ink with the number of the individual pollen parent, the date and time the enclosure was secured, and any useful notes. Room is left to add the date of pollen collection and necessary information about the future seed parent it will pollinate.

Pollen release is fairly rapid inside the bags, and after two days to a week the limbs may be removed and dried in a cool dark place, unless the bags are placed too early or the pollen parent develops very slowly. To inspect the progress of pollen release, a flashlight is held behind the bag at night and the silhouettes of the opening flowers are easily seen. In some cases, clear nylon windows are in stalled with silicon glue for greater visibility. When flowering is at its peak and many flowers have just opened, collection is completed, and the limb, with its bag attached, is cut. If the limb is cut too early, the flowers will not have shed any pollen; if the bag remains on the plant too long, most of the pollen will be dropped inside the bag where heat and moisture will destroy it. When flowering is at its peak, millions of pollen grains are released and many more flowers will open after the limbs are collected. The bags are collected early in the morning before the sun has time to heat them up. The bags and their contents are dried in a cool dark place to avoid mold and pollen spoilage. If pollen becomes moist, it will germinate and spoil, therefore dry storage is imperative.

After the staminate limbs have dried and pollen re lease has stopped, the bags are shaken vigorously, allowed to settle, and carefully untied. The limbs and loose flowers are removed, since they are a source of moisture that could promote mold growth, and the pollen bags are re sealed. The bags may be stored as they are until the seed parent is ready for pollination, or the pollen may be re moved and stored in cool, dry, dark vials for later use and hand application. Before storing pollen, any other plant parts present are removed with a screen. A piece of fuel filter screening placed across the top of a mason jar works well, as does a fine-mesh tea strainer.

Now a pistillate plant is chosen as the seed parent. A pistillate flower cluster is ripe for fertilization so long as pale, slender pistils emerge from the calyxes. Withered, dark pistils protruding from swollen, resin encrusted calyxes are a sign that the reproductive peak has long passed. Cannabis plants can be successfully pollinated as soon as the first primordia show pistils and until just before harvest, but the largest yield of uniform, healthy seeds is achieved by pollinating in the peak floral stage. At this time, the seed plant is covered with thick clusters of white pistils. Few pistils are brown and withered, and resin production has just begun. This is the most receptive time for fertilization, still early in the seed plant’s life, with plenty of time remaining for the seeds to mature. Healthy, well flowered lower limbs on the shaded side of the plant are selected. Shaded buds will not heat up in the bags as much as buds in the hot sun, and this will help

protect the sensitive pistils. When possible, two terminal clusters of pistillate flowers are chosen for each pollen bag. In this way, with two pollen bags for each seed parent and two clusters of pistillate flowers for each bag, there are four opportunities to perform the cross successfully. Remember that production of viable seed requires successful pollination, fertilization and embryo development. Since interfering with any part of this cycle precludes seed development, fertilization failure is guarded against by duplicating all steps.

Before the pollen bags are used, the seed parent information is added to the pollen parent data. Included is the number of the seed parent, the date of pollination, and any comments about the phenotypes of both parents. Also, for each of the selected pistillate clusters, a tag containing the same information is made and secured to the limb below the closure of the bag. A warm, windless evening is chosen for pollination so the pollen tube has time to grow before sunrise. After removing most of the shade leaves from the tips of the limbs to be pollinated, the pollen is tapped away from the mouth of the bag. The bag is then carefully opened and slipped over two inverted limb tips, taking care not to release any pollen, and tied securely with an expandable band. The bag is shaken vigorously, so the pollen will be evenly dispersed throughout the bag, facilitating complete pollination. Fresh bags are sometimes used, either charged with pollen prior to being placed over the limb tip, or injected with pollen, using a large syringe or atomizer, after the bag is placed. However, the risk of accidental pollination with injection is higher.

If only a small quantity of pollen is available it may be used more sparingly by diluting with a neutral powder such as flour before it is used. When pure pollen is used, many pollen grains may land on each pistil when only one is needed for fertilization. Diluted pollen will go further and still produce high fertilization rates. Diluting 1 part pollen with 10 to 100 parts flour is common. Powdered fungicides can also be used since this helps retard the growth of molds in the maturing, seeded, floral clusters.

The bags may remain on the seed parent for sometime; seeds usually begin to develop within a few days, buttheir development will be retarded by the bags. The propagator waits three full sunny days, then carefully removes and sterilizes or destroys the bags. This way there is little chance of stray pollination. Any viable pollen that failed to pollinate the seed parent will germinate in the warm moist bag and die within three days, along with many of the unpollinated pistils. In particularly cool or overcast conditions a week may be necessary, but the bag is removed at the earliest safe time to ensure proper seed development without stray pollinations. As soon as the bag is removed, the calyxes begin to swell with seed, indicating successful fertilization. Seed parents then need good irrigation or development will be retarded, resulting in small, immature, and nonviable seeds. Seeds develop fastest in

warm weather and take usually from two to four weeks to mature completely. In cold weather seeds may take up to two months to mature. If seeds get wet in fall rains, they may sprout. Seeds are removed when the calyx begins to dry up and the dark shiny perianth (seed coat) can be seen protruding from the drying calyx. Seeds are labeled and stored in a cool, dark, dry place, This is the method employed by breeders to create seeds of known parentage used to study and improve Cannabis genetics.

Seed Selection

Nearly every cultivated Cannabis plant, no matter what its future, began as a germinating seed; and nearly all Cannabis cultivators, no matter what their intention, start with seeds that are gifts from a fellow cultivator or extracted from imported shipments of marijuana. Very little true control can be exercised in seed selection unless the cultivator travels to select growing plants with favorable characteristics and personally pollinate them. This is not possible for most cultivators or researchers and they usually rely on imported seeds. These seeds are of unknown parentage, the product of natural selection or of breeding by the original farmer, Certain basic problems affect the genetic purity and predictability of collected seed.

1 - If a Cannabis sample is heavily seeded, then the majority of the male plants were allowed to mature and release pollen, Since Cannabis is wind-pollinated, many pollen parents (including early and late maturing staminate and hermaphrodite plants) will contribute to the seeds in any batch of pistillate flowers. If the seeds are all taken from one flower cluster with favorable characteristics, then at least the pistillate or seed parent is the same for all those seeds, though the pollen may have come from many different parents. This creates great diversity in offspring.

2 - In very lightly seeded or nearly sinsemilla Cannabis, pollination has largely been prevented by the removal of staminate parents prior to the release of pollen. The few seeds that do form often result from pollen from hermaphrodite plants that went undetected by the farmer, or by random wind-borne pollen from wild plants or a nearby field. Hermaphrodite parents often produce hermaphrodite offspring and this may not be desirable.

3 - Most domestic Cannabis strains are random hybrids. This is the result of limited selection of pollen parents, impure breeding conditions, and lack of adequate space to isolate pollen parents from the remainder of the crop.

When selecting seeds, the propagator will frequently look for seed plants that have been carefully bred locally by another propagator. Even if they are hybrids

there is a better chance of success than with imported seeds, pro vided certain guidelines are followed:

1 - The dried seeded flower clusters are free of staminate flowers that might have caused hermaphrodite pollinations.

2 - The flowering clusters are tested for desirable traits and seeds selected from the best.

3 - Healthy, robust seeds are selected. Large, dark seeds are best; smaller, paler seeds are avoided since these are usually less mature and less viable.

4 - If accurate information is not available about the pollen parent, then selection proceeds on common sense and luck. Mature seeds with dried calyxes in the basal portions of the floral clusters along the main stems occur in the earliest pistillate flowers to appear and must have been pollinated by early-maturing pollen parents. These seeds have a high chance of producing early-maturing offspring. By contrast, mature seeds selected from the tips of floral clusters, often surrounded by immature seeds, are formed in later-appearing pistillate flowers. These flowers were likely pollinated by later-maturing staminate or hermaphrodite pollen parents, and their seeds should mature later and have a greater chance of producing hermaphrodite off spring. The pollen parent also exerts some influence on the appearance of the resulting seed. If seeds are collected from the same part of a flower cluster and selected for similar size, shape, color, and perianth patterns, then it is more likely that the pollinations represent fewer different gene pools and will produce more uniform offspring.

5 - Seeds are collected from strains that best suit the locality; these usually come from similar climates and latitudes. Seed selection for specific traits is discussed in detail in Chapter III.

6 - Pure strain seeds are selected from crosses between parents of the same origin.

7 - Hybrid seeds are selected from crosses between pure strain parents of different origins.

8 - Seeds from hybrid plants, or seeds resulting from pollination by hybrid plants, are avoided, since these will not reliably reproduce the phenotype of either parent.

Seed stocks are graded by the amount of control exerted by the collector in selecting the parents. Grade #1 - Seed parent and pollen parent are known and there is absolutely no possibility that the seeds resulted from pollen contamination.

Grade #2 - Seed parent is known but several known staminate or hermaphrodite pollen parents are involved. Grade #3 - Pistillate parent is known and pollen parents are unknown.

Grade #4 - Neither parent is known, but the seeds are collected from one floral cluster, so the pistillate seed parent age traits may be characterized.

Grade #5 - Parentage is unknown but origin is certain, such as seeds collected from the bottom of a bag of imported Cannabis.

Grade #6 - Parentage and origin are unknown.

Asexual Propagation

Asexual propagation (cloning) allows the preservation of genotype because only normal cell division (mitosis) occurs during growth and regeneration. The vegetative (non-reproductive) tissue of Cannabis has 10 pairs of chromosomes in the nucleus of each cell. This is known as the diploid (2n) condition where 2n = 20 chromosomes. During mitosis every chromosome pair replicates and one of the two identical sets of chromosome pairs migrates to each daughter cell, which now has a genotype identical to the mother cell. Consequently, every vegetative cell in a Cannabis plant has the same genotype and a plant resulting from asexual propagation will have the same genotype as the mother plant and will, for all practical purposes, develop identically under the same environmental conditions.

In Cannabis, mitosis takes place in the shoot apex (meristem), root tip meristems, and the meristematic cambium layer of the stalk. A propagator makes use of these meristematic areas to produce clones that will grow and be multiplied. Asexual propagation techniques such as cuttage, layerage, and division of roots can ensure identical populations as large as the growth and development of the parental material will permit. Clones can be produced from even a single cell, because every cell of the plant possesses the genetic information necessary to regenerate a complete plant.

Asexual propagation produces clones which perpetuate the unique characteristics of the parent plant. Because of the heterozygous nature of Cannabis, valuable traits may be lost by sexual propagation that can be preserved and multiplied by cloning. Propagation of nearly identical populations of all-pistillate, fast growing, evenly maturing Cannabis is made possible through

cloning. Any agricultural or environmental influences will affect all the members of that clone equally.

The concept of clone does not mean that all members of the clone will necessarily appear identical in all characteristics. The phenotype that we observe in an individual is influenced by its surroundings. Therefore, members of the clone will develop differently under varying environmental conditions. These influences do not affect genotype and therefore are not permanent. Cloning theoretically can pre serve a genotype forever. Vigor may slowly decline due to poor selection of clone material or the constant pressure of disease or environmental stress, but this trend will re verse if the pressures are removed. Shifts in genetic composition occasionally occur during selection for vigorous growth. However, if parental strains are maintained by in frequent cloning this is less likely. Only mutation of a gene in a vegetative cell that then divides and passes on the mutated gene will permanently affect the genotype of the clone. If this mutated portion is cloned or reproduced sexually, the mutant genotype will be further replicated. Mutations in clones usually affect dominance relations and are therefore noticed immediately. Mutations may be induced artificially (but without much predictability) by treating meristematic regions with X-rays, colchicine, or other mutagens.

The genetic uniformity provided by clones offers a control for experiments designed to quantify the subtle effects of environment and cultural techniques. These subtleties are usually obscured by the extreme diversity resulting from sexual propagation. However, clonal uniformity can also invite serious problems. If a population of clones is subjected to sudden environmental stress, pests, or disease for which it has no defense, every member of the clone is sure to be affected and the entire population may be lost. Since no genetic diversity is found within the clone, no adaptation to new stresses can occur through recombination of genes as in a sexually propagated population.

In propagation by cuttage or layerage it is only necessary for a new root system to form, since the meristematic shoot apex comes directly from the parental plant. Many stem cells, even in mature plants, have the capability of producing adventitious roots. In fact, every vegetative cell in the plant contains the genetic information needed for an entire plant. Adventitious roots appear spontaneously from stems and old roots as opposed to systemic roots which appear along the developing root system originating in the embryo. In humid conditions (as in the tropics or a green house) adventitious roots occur naturally along the main stalk near the ground and along limbs where they droop and touch the ground.

Rooting

A knowledge of the internal structure of the stem is helpful in understanding the origin of adventitious roots.

The development of adventitious roots can be broken down into three stages: (1) the initiation of meristematic cells located just outside and between the vascular bundles (the root initials), (2) the differentiation of these meristematic cells into root primordia, and (3) the emergence and growth of new roots by rupturing old stem tissue and establishing vascular connections with the shoot.

As the root initials divide, the groups of cells take on the appearance of a small root tip. A vascular system forms with the adjacent vascular bundles and the root continues to grow outward through the cortex until the tip emerges from the epidermis of the stem. Initiation of root growth usually begins within a week and young roots appear within four weeks. Often an irregular mass of white cells, termed callus tissue, will form on the surface of the stem adjacent to the areas of root initiation. This tissue has no influence on root formation. However, it is a form of regenerative tissue and is a sign that conditions are favorable for root initiation.

The physiological basis for root initiation is well understood and allows many advantageous modifications of rooting systems. Natural plant growth substances such as auxins, cytokinins, and gibberellins are certainly responsible for the control of root initiation and the rate of root formation. Auxins are considered the most influential. Auxins and other growth substances are involved in the control of virtually all plant processes: stem growth, root formation, lateral bud inhibition, floral maturation, fruit development, and determination of sex. Great care is exercised in application of artificial growth substances so that detrimental conflicting reactions in addition to rooting do not occur. Auxins seem to affect most related plant species in the same way, but the mechanism of this action is not yet fully understood.

Many synthetic compounds have been shown to have auxin activity and are commercially available, such as napthaleneacetic acid (NAA), indolebutyric acid (IBA), and 2,4-dichlorophenoxyacetic acid (2,4 DPA), but only indoleacetic acid has been isolated from plants. Naturally occurring auxin is formed mainly in the apical shoot men stem and young leaves. It moves downward after its formation at the growing shoot tip, but massive concentrations of auxins in rooting solutions will force travel up the vascular tissue. Knowledge of the physiology of auxins has led to practical applications in rooting cuttings. It was shown originally by Went and later by Thimann and Went that auxins promote adventitious root formation in stem cuttings. Since application of natural or synthetic auxin seems to stimulate adventitious root formation in many plants, it is assumed that auxin levels are associated with the formation of root initials. Further research by Warmke and Warmke (1950) suggested that the levels of auxin may determine whether adventitious roots or shoots are formed, with high auxin levels promoting root growth and low levels favoring shoots.

Cytokinins are chemical compounds that stimulate cell growth. In stem cuttings, cytokinins suppress root growth and stimulate bud growth. This is the opposite of

the reaction caused by auxins, suggesting that a natural balance of the two may be responsible for regulating nor mal plant growth. Skoog discusses the use of solutions of equal concentrations of auxins and cytokinins to pro mote the growth of undifferentiated callus tissues. This may provide a handy source of undifferentiated material for cellular cloning.

Although Cannabis cuttings and layers root easily, variations in rootability exist and old stems may resist rooting. Selection of rooting material is highly important. Young, firm, vegetative shoots, 3 to 7 millimeters (1/8 to ¼ inch) in diameter, root most easily. Weak, unhealthy plants are avoided, along with large woody branches and reproductive tissues, since these are slower to root. Stems of high carbohydrate content root most easily. Firmness is a sign of high carbohydrate levels in stems but may be con fused with older woody tissue. An accurate method of determining the carbohydrate content of cuttings is the iodine starch test. The freshly cut ends of a bundle of cuttings are immersed in a weak solution of iodine in potassium iodide. Cuttings containing the highest starch content stain the darkest; the samples are rinsed and sorted accordingly. High nitrogen content cuttings seem to root more poorly than cuttings with medium to low nitrogen content. Therefore, young, rapidly-growing stems of high nitrogen and low carbohydrate content root less well than slightly older cuttings. For rooting, sections are selected that have ceased elongating and are beginning radial growth. Staminate plants have higher average levels of carbohydrates than pistillate plants, while pistillate plants exhibit higher nitrogen levels. It is unknown whether sex influences rooting, but cuttings from vegetative tissue are taken just after sex determination while stems are still young. For rooting cloning stock or parental plants, the favorable balance (low nitrogen-to-high carbohydrate) is achieved in several ways:

1 - Reduction of the nitrogen supply will slow shoot growth and allow time for carbohydrates to accumulate. This can be accomplished by leaching (rinsing the soil with large amounts of fresh water), withholding nitrogenous fertilizer, and allowing stock plants to grow in full sun light. Crowding of roots reduces excessive vegetative growth and allows for carbohydrate accumulation.

2 - Portions of the plant that are most likely to root are selected. Lower branches that have ceased lateral growth and begun to accumulate starch are the best. The carbohydrate-to-nitrogen ratio rises as you move away from the tip of the limb, so cuttings are not made too short.

3 - Etiolation is the growth of stem tissue in total darkness to increase the possibility of root initiation. Starch levels drop, strengthening tissues and fibers begin to soften, cell wall thickness decreases, vascular tissue is diminished, auxin levels rise, and undifferentiated tissue begins to form. These conditions are very

conducive to the initiation of root growth. If the light cycle can be con trolled, whole plants can be subjected to etiolation, but usually single limbs are selected for cloning and wrapped for several inches just above the area where the cutting will be taken. This is done two weeks prior to rooting. The etiolated end may then be unwrapped and inserted into the rooting medium. Various methods of layers and cuttings rooted below soil level rely in part on the effects of etiolation.

4 - Girdling a stem by cutting the phloem with a knife or crushing it with a twisted wire may block the downward mobility of carbohydrates and auxin and rooting cofactors, raising the concentration of these valuable components of root initiation above the girdle.

Making Cuttings

Cuttings of relatively young vegetative limbs 10 to 45 centimeters (4 to 18 inches) are made with a sharp knife or razor blade and immediately placed in a container of clean, pure water so the cut ends are well covered. It is essential that the cuttings be placed in water as soon as they are removed or a bubble of air (embolism) may enter the cut end and block the transpiration stream in the cutting, causing it to wilt. Cuttings made under water avoid the possibility of an embolism. If cuttings are exposed to the air they are cut again before being inserted into the rooting medium.

The medium should be warm and moist before cut tings are removed from the parental plant. Rows of holes are made in the rooting medium with a tapered stick, slightly larger in diameter than the cutting, leaving at least 10 centimeters (4 inches) between each hole. The cuttings are removed from the water, the end to be rooted treated with growth regulators and fungicides (such as Rootone F or Hormex), and each cutting placed in its hole. The cut end of the shoot is kept at least 10 centimeters (4 inches) from the bottom of the medium. The rooting medium is lightly tamped around the cutting, taking care not to scrape off the growth regulators. During the first few days the cuttings are checked frequently to make sure every thing is working properly. The cuttings are then watered with a mild nutrient solution once a day.

Hardening-off

The cuttings usually develop a good root system and will be ready to transplant in three to six weeks. At this time the hardening-off process begins, preparing the delicate cuttings for a life in bright sunshine. The cuttings are removed and transplanted to a sheltered spot such as a greenhouse until they begin to grow

on their own. It is necessary to water them with a dilute nutrient solution or feed with finished compost as soon as the hardening-off process begins. Young roots are very tender and great care is necessary to avoid damage. When vegetative cuttings are placed outside under the prevailing photoperiod they will react accordingly. If it is not the proper time of the year for the cuttings to grow and mature properly (near harvest time, for example) or if it is too cold for them to be put out, then they may be kept in a vegetative condition by supplementing their light to increase daylength. Alternatively they may be induced to flower indoors under artificial conditions.

After shoots are selected and prepared for cloning, they are treated and placed in the rooting medium. Since the discovery in 1984 that auxins such as IAA stimulate the production of adventitious roots, and the subsequent discovery that the application of synthetic auxins such as NAA increase the rate of root production, many new techniques of treatment have appeared. It has been found that mixtures of growth regulators are often more effective than one alone. IAA and NAA a—e often combined with a small percentage of certain phenoxy compounds and fungicides in commercial preparations. Many growth regulators deteriorate rapidly, and fresh solutions are made up as needed. Treatments with vitamin B1 (thiamine) seem to help roots grow, but no inductive effect has been noticed. As soon as roots emerge, nutrients are necessary; the shoot cannot maintain growth for long on its own reserves. A complete complement of nutrients in the rooting medium certainly helps root growth; nitrogen is especially beneficial. Cuttings are extremely susceptible to fungus attack, and conditions conducive to rooting are also favorable to the growth of fungus. "Cap tan " is a long-lasting fungicide that is sometimes applied in powdered form along with growth regulators. This is done by rolling the basal end of the cutting in the powder before placing it in the rooting medium.

Oxygen and Rooting

The initiation and growth of roots depends upon atmospheric oxygen. If oxygen levels are low, shoots may fail to produce roots and rooting will certainly be inhibited. It is very important to select a light, well-aerated rooting medium. In addition to natural aeration from the atmosphere, rooting media may be enriched with oxygen (02) gas; enriched rooting solutions have been shown to increase rooting in many plant species. No threshold for damage by excess oxygenation has been determined, although excessive oxygenation could displace carbon dioxide which is also vital for proper root initiation and growth. If oxygen levels are low, roots will form only near the surface of the medium, whereas with adequate oxygen levels, roots will tend to form along the entire length of the implanted shoot, especially at the cut end.

Oxygen enrichment of rooting media is fairly simple. Since shoot cuttings must be constantly wetted to ensure proper rooting, aeration of the rooting media may be facilitated by aerating the water used in irrigation. Mist systems achieve this

automatically because they deliver a fine mist (high in dissolved oxygen) to the leaves, from where much of it runs off into the soil, aiding rooting. Oxygen enrichment of irrigation water is accomplished by installing an aerator in the main water line so that atmospheric oxygen can be absorbed by the water. An increase in dissolved oxygen of only 20 parts per million may have a great influence on rooting. Aeration is a convenient way to add oxygen to water as it also adds carbon dioxide from the atmosphere. Air from a small pump or bottled oxygen may also be supplied directly to the rooting media through tiny tubes with pin holes, or through a porous stone such as those used to aerate aquariums.

Rooting Media

Water is a common medium for rooting. It is inexpensive, disperses nutrients evenly, and allows direct observation of root development. However, several problems arise. A water medium allows light to reach the submerged stem, delaying etiolation and slowing root growth. Water also promotes the growth of water molds and other fungi, sup ports the cutting poorly, and restricts air circulation to the young roots. In a well aerated solution, roots will appear in great profusion at the base of the stem, while in a poorly aerated or stagnant solution only a few roots will form at the surface, where direct oxygen exchange occurs. If rootings are made in pure water, the solution might be replaced regularly with tap water, which should contain sufficient oxygen for a short period. If nutrient solutions are used, a system is needed to oxygenate the solution. The nutrient solution does become concentrated by evaporation, and this is watched. Pure water is used to dilute rooting solutions and refill rooting containers.

Soil Treatment

Solid media provide anchors for cuttings, plenty of darkness to promote etiolation and root growth, and sufficient air circulation to the young roots. A high-quality soil with good drainage such as that used for seed germination is often used but the soil must be carefully sterilized to prevent the growth of harmful bacteria and fungus. A small amount of soil can easily be sterilized by spreading it out on a cookie sheet and heating it in an oven set at "low," approximately 820 C (180~ F), for thirty minutes. This kills most harmful bacteria and fungus as well as nematodes, in sects and most weed seeds. Overheating the soil will cause the breakdown of nutrients and organic complexes and the formation of toxic compounds. Large amounts of soil may be treated by chemical fumigants. Chemical fumigation avoids the breakdown of organic material by heat and may result in a better rooting mix. Formaldehyde is an excellent fungicide and kills some weed seeds, nematodes, and in sects. One gallon of commercial formalin (40% strength) is mixed with 50 gallons of water and slowly applied until each cubic foot of soil absorbs 2-4 quarts of solution. Small containers are sealed with plastic bags; large flats and plots are covered with polyethylene sheets. After 24 hours the seal is removed and the soil is allowed to dry for two weeks or until the odor of formaldehyde is no longer present. The treated soil is drenched with

water prior to use. Fumigants such as formaldehyde, methyl bromide or other lethal gases are very dangerous and cultivators use them only outside with appropriate protection for themselves.

It is usually much simpler and safer to use an artificial sterile medium for rooting. Vermiculite and perlite are often used in propagation because of their excellent drain age and neutral pH (a balance between acidity and alkalinity). No sterilization is needed because both products are manufactured at high heat and contain no organic material. It has been found that a mixture of equal portions of medium and large grade vermiculite or perlite promotes the greatest root growth. This results from increased air circulation around the larger pieces. A weak nutrient solution, including micro-nutrients, is needed to wet the medium, because little or no nutrient material is supplied by these artificial media. Solutions are checked for pH and corrected to neutral with agricultural lime, dolomite lime, or oyster shell lime.

Layering

Layering is a process in which roots develop on a stem while it remains attached to, and nutritionally sup ported by the parent plant. The stem is then detached and the meristematic tip becomes a new individual, growing on its own roots, termed a layer. Layering differs from cutting because rooting occurs while the shoot is still attached to the parent. Rooting is initiated in layering by various stem treatments which interrupt the downward flow of photosynthates (products of photosynthesis) from the shoot tip. This causes the accumulation of auxins, carbohydrates and other growth factors. Rooting occurs in this treated area even though the layer remains attached to the parent. Water and mineral nutrients are supplied by the parent plant because only the phloem has been interrupted; the xylem tissues connecting the shoot to the parental roots remain intact (see illus. 1, page 29). In this manner, the propagator can overcome the problem of keeping a severed cutting alive while it roots, thus greatly in creasing the chances of success. Old woody reproductive stems that, as cuttings, would dry up and die, may be rooted by layering. Layering can be very time-consuming and is less practical for mass cloning of parental stock than removing and rooting dozens of cuttings. Layering, however, does give the small-scale propagator a high-success alternative which also requires less equipment than cuttings.

Techniques of Layering

Almost all layering techniques rely on the principle of etiolation. Both soil layering and air layering involve depriving the rooting portion of the stem of light, promoting rooting. Root-promoting substances and fungicides prove beneficial, and they are usually applied as a spray or powder. Root formation on layers depends on constant moisture, good air circulation and moderate temperatures at the site of rooting.

Soil Layering

Soil layering may be performed in several ways. The most common is known as tip layering. A long, supple vegetative lower limb is selected for layering, carefully bent so it touches the ground, and stripped of leaves and small shoots where the rooting is to take place. A narrow trench, 6 inches to a foot long and 2 to 4 inches deep, is dug parallel to the limb, which is placed along the bottom of the trench, secured with wire or wooden stakes, and buried with a small mound of soil. The buried section of stem may be girdled by cutting, crushed with a loop of wire, or twisted to disrupt the phloem tissue and cause the accumulation of substances which promote rooting. It may also be treated with growth regulators at this time.

Serpentine layering may be used to create multiple layers along one long limb. Several stripped sections of the limb are buried in separate trenches, making sure that at least one node remains above ground between each set of roots to allow shoots to develop. The soil surrounding the stem is kept moist at all times and may require wetting several times a day. A small stone or stick is inserted under each exposed section of stem to prevent the lateral shoot buds rotting from constant contact with the moist soil surface. Tip layers and serpentine layers may be started in small containers placed near the parental plant. Rooting usually begins within two weeks, and layers may be re moved with a sharp razor or clippers after four to six weeks. If the roots have become well established, transplanting may be difficult without damaging the tender root system. Shoots on layers continue to grow under the same conditions as the parent, and less time is needed for the clone to acclimatize or harden-off and begin to grow on its own than with cuttings.

In air layering, roots form on the aerial portions of stems that have been girdled, treated with growth regulators, and wrapped with moist rooting media. Air layering is an ancient form of propagation, possibly invented by the Chinese. The ancient technique of goo tee uses a ball of clay or soil plastered around a girdled stem and held with a wrap of fibers. Above this is suspended a small container of water (such as a bamboo section) with a wick to the wrapped gootee; this way the gootee remains moist.

The single most difficult problem with air layers is the tendency for them to dry out quickly. Relatively small amounts of rooting media are used, and the position on aerial parts of the plant exposes them to drying winds and sun. Many wraps have been tried, but the best seems to be clear polyethylene plastic sheeting which allows oxygen to enter and retains moisture well. Air layers are easiest to make in greenhouses where humidity is high, but they may also be used outside as long as they are kept moist and don’t freeze. Air layers are most useful to the amateur propagator and breeder because they take up little space and allow the efficient cloning of many individuals.

Making an Air Layer

A recently sexed young limb 3-10 mm (1/8 to 3/8 inch) in diameter is selected. The site of the layer is usually a spot 30 centimeters (12 inches) or more from the limb tip. Unless the stem is particularly strong and woody, it is splinted by positioning a 30 centimeter (12 inch) stick of approximately the same diameter as the stem to be layered along the bottom edge of the stem. This splint is tied in place at both ends with a piece of elastic plant-tie tape. This enables the propagator to handle the stem more confidently. An old, dry Cannabis stem works well as a splint. Next, the stem is girdled between the two ties with a twist of wire or a diagonal cut. After girdling, the stem is sprayed or dusted with a fungicide and growth regulator, surrounded with one or two handfuls of unmilled sphagnum moss, and wrapped tightly with a small sheet of clear polyethylene film (4-6 mil). The film is tied securely at each end, tightly enough to make a waterproof seal but not so tight that the phloem tissues are crushed. If the phloem is crushed, compounds necessary for rooting will accumulate outside of the medium and rooting will be slowed. Plastic florist's tape or electrician's tape works well for sealing air layers. Although polyethylene film retains moisture well, the moss will dry out eventually and must be remoistened periodically. Unwrapping each layer is impractical and would disturb the roots, so a hypodermic syringe is used to inject water, nutrients, fungicides, and growth regulators. If the layers become too wet the limb rots. Layers are checked regularly by injecting water until it squirts out and then very lightly squeezing the medium to remove any extra water. Heavy layers on thin limbs are supported by tying them to a large adjacent limb or a small stick anchored in the ground. Rooting begins within two weeks and roots will be visible through the clear plastic within four weeks. When the roots appear adequately developed, the layer is removed, carefully unwrapped, and transplanted with the moss and the splint intact. The layer is watered well and placed in a shady spot for a few days to allow the plant to harden-off and adjust to living on its own root system. It is then placed in the open. In hot weather, large leaves are removed from the shoot before removing the layer to prevent excessive transpiration and wilting.

Layers develop fastest just after sexual differentiation. Many layers may be made of staminate plants in order to save small samples of them for pollen collection and to conserve space. By the time the pollen parents begin to flower profusely, the layers will be rooted and may be cut and removed to an isolated area. Layers taken from pistil late plants are used for breeding, or saved and cloned for the following season.

Layers often seem rejuvenated when they are re moved from the parent plant and begin to be supported by their own root systems. This could mean that a clone will continue to grow longer and mature later than its parent under the same conditions. Layers removed from old or seeded parents will continue to produce new calyxes and pistils instead of completing the life cycle along with the parents. Rejuvenated layers are useful for off-season seed production.

Grafting

Intergeneric grafts between Cannabis and Humulus (hops) have fascinated researchers and cultivators for decades. Warmke and Davidson (1943) claimed that Humbles tops grafted upon Cannabis roots produced ". . . as much drug as leaves from intact hemp plants, even though leaves from intact hop plants are completely nontoxic." According to this research, the active ingredient of Cannabis was being produced in the roots and transported across the graft to the Humulus tops. Later research by Crombie and Crombie (1975) entirely disproves this theory. Grafts were made between high and low THC strains of Cannabis as well as intergeneric grafts between Cannabis and Humulus, Detailed chromatographic analysis was performed on both donors for each graft and their control populations. The results showed ". . . no evidence of transport of inter mediates or factors critical to cannabinoid formation across the grafts."

Grafting of Cannabis is very simple. Several seedlings can be grafted together into one to produce very interesting specimen plants. One procedure starts by planting one seed ling each of several separate strains close together in the same container, placing the stock (root plant) for the cross in the center of the rest. When the seedlings are four weeks old they are ready to be grafted. A diagonal cut is made approximately half-way through the stock stem and one of the scion (shoot) seedlings at the same level. The cut portions are slipped together such that the inner cut surfaces are touching. The joints are held with a fold of cellophane tape. A second scion from an adjacent seedling may be grafted to the stock higher up the stem. After two weeks, the unwanted portions of the grafts are cut away. Eight to twelve weeks are needed to complete the graft, and the plants are maintained in a mild environment at all times. As the graft takes, and the plant begins to grow, the tape falls off.

Pruning

Pruning techniques are commonly used by Cannabis cultivators to limit the size of their plants and promote branching. Several techniques are available, and each has its advantages and drawbacks. The most common method is meristem pruning or stem tip removal. In this case the growing tip of the main stalk or a limb is removed at approximately the final length desired for the stalk or limb. Below the point of removal, the next pair of axial growing tips begins to elongate and form two new limbs. The growth energy of one stem is now divided into two, and the diffusion of growth energy results in a shorter plant which spreads horizontally.

Auxin produced in the tip meristem travels down the stem and inhibits branching. When the meristem is re moved, the auxin is no longer produced and branching may proceed uninhibited. Plants that are normally very tall and stringy can be kept short and bushy by meristem pruning. Removing meristems also removes the newly formed tissues near the meristem that react to changing environmental

stimuli and induce flowering. Pruning during the early part of the growth cycle will have little effect on flowering, but plants that are pruned late in life, supposedly to promote branching and floral growth, will often flower late or fail to flower at all. This happens because the meristemic tissue responsible for sensing change has been removed and the plant does not measure that it is the time of the year to flower. Plants will usually mature fastest if they are allowed to grow and develop without interference from pruning. If late maturation of Cannabis is desired, then extensive pruning may work to delay flowering. This is particularly applicable if a staminate plant from an early maturing strain is needed to pollinate a late-maturing pistil late plant. The staminate plant is kept immature until the pistillate plant is mature and ready to be pollinated. When the pistillate plant is receptive, the staminate plant is allowed to develop flowers and release pollen.

Other techniques are available for limiting the size and shape of a developing Cannabis plant without removing meristematic tissues. Trellising is a common form of modification and is achieved in several ways. In many cases space is available only along a fence or garden row. Posts 1 to 2 meters (3 to 6 feet) long may be driven into the ground 1 to 3 meters (3 to 10 feet) apart and wires stretched between them at 30 to 45 centimeters (12 to 18 inches) intervals, much like a wire fence or grape trellis. Trellises are ideally oriented on an east-west axis for maxi mum sun exposure. Seedlings or pistillate clones are placed between the posts, and as they grow they are gradually bent and attached to the wire. The plant continues to grow upward at the stem tips, but the limbs are trained to grow horizontally. They are spaced evenly along the wires by hooking the upturned tips under the wire when they are 15 to 30 centimeters (6 to 12 inches) long. The plant grows and spreads for some distance, but it is never allowed to grow higher than the top row of wire. When the plant be gins to flower, the floral clusters are allowed to grow up ward in a row from the wire where they receive maximum sun exposure. The floral clusters are supported by the wire above them, and they are resistant to weather damage. Many cultivators feel that trellised plants, with increased sun exposure and meristems intact, produce a higher yield than freestanding unpruned or pruned plants. Other growers feel that any interference with natural growth patterns limits the ultimate size and yield of the plant.

Another method of trellising is used when light exposure is especially crucial, as with artificial lighting systems. Plants are placed under a horizontal or slightly slanted flat sheet of 2 to 5 centimeters (1 to 2 inches) poultry netting which is suspended on a frame 30 to 60 centimeters (12 to 24 inches) from the soil surface perpendicular to the direction of incoming light or to the lowest path of the sun. The seedlings or clones begin to grow through the netting al-‘ most immediately, and the meristems are pushed back down under the netting, forcing them to grow horizon tally outward. Limbs are trained so that the mature plant will cover the entire frame evenly. Once again, when the plant begins to flower, the floral clusters are allowed to grow upward through the wire as they reach for the light. This might prove to be a feasible commercial cultivation technique, since

the flat beds of floral clusters could be mechanically harvested. Since no meristem tissues are re moved, growth and maturation should proceed on schedule. This system also provides maximum light exposure for all the floral clusters, since they are growing from a plane perpendicular to the direction of light.

Sometimes limbs are also tied down, or crimped and bent to limit height and promote axial growth without meristem removal. This is a particularly useful technique for greenhouse cultivation, where plants often reach the roof or walls and burn or rot from the intense heat and condensation of water on the inside of the greenhouse. To prevent rotting and burning while leaving enough room for floral clusters to form, the limbs are bent at least 60 centimeters (24 inches) beneath the roof of the green house. Tying plants over allows more light to strike the plant, promoting axial growth. Crimping stems and bending them over results in more light exposure as well as inhibiting the flow of auxin down the stem from the tip. Once again, as with meristem removal, this promotes axial growth.

Limbing is another common method of pruning Cannabis plants. Many small limbs will usually grow from the bottom portions of the plant, and due to shading they re main small and fail to develop large floral clusters. If these atrophied lower limbs are removed, the plant can devote more of its floral energies to the top parts of the plant with the most sun exposure and the greatest chance of pollination. The question arises of whether removing entire limbs constitutes a shock to the growing plant, possibly limiting its ultimate size. It seems in this case that shock is minimized by removing entire limbs, including proportional amounts of stems, leaves, meristems, and flowers; this probably results in less metabolic imbalance than if only flowers, leaves, or meristems were removed. Also, the lower limbs are usually very small and seem of little significance in the metabolism of the total plant. In large plants, many limbs near the central stalk also become shaded and atrophied and these are also sometimes removed in an effort to increase the yield of large floral clusters on the sunny exterior margins.

Leafing is one of the most misunderstood techniques of drug Cannabis cultivation. In the mind of the cultivator, several reasons exist for removing leaves. Many feel that large shade leaves draw energy from the flowering plant, and therefore the flowering clusters will be smaller. It is felt that by removing the leaves, surplus energy will be available, and large floral clusters will be formed. Also, some feel that inhibitors of flowering, synthesized in the leaves during the long noninductive days of summer, may be stored in the older leaves that were formed during the noninductive photoperiod. Possibly, if these inhibitor-laden leaves are removed, the plant will proceed to flower, and maturation will be accelerated. Large leaves shade the inner portions of the plant, and small atrophied floral clusters may begin to develop if they receive more light.

In actuality, few if any of the theories behind leafing give any indication of validity. Indeed, leafing possibly serves to defeat its original purpose. Large leaves have

a definite function in the growth and development of Cannabis. Large leaves serve as photosynthetic factories for the production of sugars and other necessary growth sub stances. They also create shade, but at the same time they are collecting valuable solar energy and producing foods that will be used during the floral development of the plant. Premature removal of leaves may cause stunting, because the potential for photosynthesis is reduced. As these leaves age and lose their ability to carry on photo synthesis they turn chlorotie (yellow) and fall to the ground. In humid areas care is taken to remove the yellow or brown leaves, because they might invite attack by fungus. During chlorosis the plant breaks down substances, such as chlorophylls, and translocates the molecular components to a new growing part of the plant, such as the flowers. Most Cannabis plants begin to lose their larger leaves when they enter the flowering stage, and this trend continues until senescence. It is more efficient for the plant to reuse the energy and various molecular components of existing chlorophyll than to synthesize new chlorophyll at the time of flowering. During flowering this energy is needed to form floral clusters and ripen seeds.

Removing large amounts of leaves may interfere with the metabolic balance of the plant. If this metabolic change occurs too late in the season it could interfere with floral development and delay maturation. If any floral inhibitors are removed, the intended effect of accelerating flowering will probably be counteracted by metabolic upset in the plant. Removal of shade leaves does facilitate more light reaching the center of the plant, but if there is not enough food energy produced in the leaves, the small internal floral clusters will probably not grow any larger. Leaf removal may also cause sex reversal resulting from a metabolic change.

If leaves must be removed, the petiole is cut so that at least an inch remains attached to the stalk. Weaknesses in the limb axis at the node result if the leaves are pulled off at the abscission layer while they are still green. Care is taken to see that the shriveling petiole does not invite fungus attack.

It should be remembered that, regardless of strain or environmental conditions, the plant strives to reproduce, and reproduction is favored by early maturation. This produces a situation where plants are trying to mature and reproduce as fast as possible. Although the purpose of leafing is to speed maturation, disturbing the natural progressive growth of a plant probably interferes with its rapid development.

Cannabis grows largest when provided with plentiful nutrients, sunlight, and water and left alone to grow and mature naturally. It must be remembered that any alteration of the natural life cycle of Cannabis will affect productivity. Imaginative combinations and adaptations of propagation techniques exist, based on specific situations of cultivation. Logical choices are made to direct the natural growth cycle of Cannabis to favor the timely maturation of those products sought by the cultivator, without sacrificing seed or clone production.

Chapter 3 - Genetics and Breeding of Cannabis

"The greatest service which can be rendered to any country is to add a useful plant to its culture."

- Thomas Jefferson

Genetics

Although it is possible to breed Cannabis with limited success without any knowledge of the laws of inheritance, the full potential of diligent breeding, and the line of action most likely to lead to success, is realized by breeders who have mastered a working knowledge of genetics.

As we know already, all information transmitted from generation to generation must be contained in the pollen of the staminate parent and the ovule of the pistillate parent. Fertilization unites these two sets of genetic information, a seed forms, and a new generation is begun. Both pollen and ovules are known as gametes, and the transmitted units determining the expression of a character are known as genes. Individual plants have two identical sets of genes (2n) in every cell except the gametes, which through reduction division have only one set of genes (in). Upon fertilization one set from each parent combines to form a seed (2n).

In Cannabis, the haploid (in) number of chromosomes is 10 and the diploid (2n) number of chromosomes is 20. Each chromosome contains hundreds of genes, influencing every phase of the growth and development of the plant.

If cross-pollination of two plants with a shared genetic trait (or self-pollination of a hermaphrodite) results in off spring that all exhibit the same trait, and if all subsequent (inbred) generations also exhibit it, then we say that the strain (i.e., the line of offspring derived from common ancestors) is true-breeding, or breeds

true, for that trait. A strain may breed true for one or more traits while varying in other characteristics. For example, the traits of sweet aroma and early maturation may breed true, while off spring vary in size and shape. For a strain to breed true for some trait, both of the gametes forming the offspring must have an identical complement of the genes that influence the expression of that trait. For example, in a strain that breeds true for webbed leaves, any gamete from any parent in that population will contain the gene for webbed leaves, which we will signify with the letter w. Since each gamete carries one-half (in) of the genetic complement of the offspring, it follows that upon fertilization both "leaf shape" genes of the (2n) offspring will be w. That is, the offspring, like both parents, are ww. In turn, the offspring also breed true for webbed leaves because they have only w genes to pass on in their gametes.

On the other hand, when a cross produces offspring that do not breed true (i.e., the offspring do not all resemble their parents) we say the parents have genes that segregate or are hybrid. Just as a strain can breed true for one or more traits, it can also segregate for one or more traits; this is often seen. For example, consider a cross where some of the offspring have webbed leaves and some have normal compound-pinnate leaves. (To continue our system of notation we will refer to the gametes of plants with compound-pinnate leaves as W for that trait. Since these two genes both influence leaf shape, we assume that they are related genes, hence the lower-case w and upper-case W notation instead of w for webbed and possibly P for pinnate.) Since the gametes of a true-breeding strain must each have the same genes for the given trait, it seems logical that gametes which produce two types of offspring must have genetically different parents.

Observation of many populations in which offspring differed in appearance from their parents led Mendel to his theory of genetics. If like only sometimes produces like, then what are the rules which govern the outcome of these crosses? Can we use these rules to predict the outcome of future crosses?

Assume that we separate two true-breeding populations of Cannabis, one with webbed and one with compound-pinnate leaf shapes. We know that all the gametes produced by the webbed-leaf parents will contain genes for leaf-shape w and all gametes produced by the compound-pinnate individuals will have W genes for leaf shape. (The offspring may differ in other characteristics, of course.)

If we make a cross with one parent from each of the true-breeding strains, we will find that 100% of the off spring are of the compound-pinnate leaf phenotype. (The expression of a trait in a plant or strain is known as the phenotype.) What happened to the genes for webbed leaves contained in the webbed leaf parent? Since we know that there were just as many w genes as W genes combined in the offspring, the W gene must mask the expression of the w gene. We term the W gene the dominant gene and say that the trait of compound-pinnate leaves is

dominant over the recessive trait of webbed leaves. This seems logical since the normal phenotype in Cannabis has compound-pinnate leaves. It must be remembered, however, that many useful traits that breed true are recessive. The true-breeding dominant or recessive condition, WW or ww, is termed the homozygous condition; the segregating hybrid condition wW or Ww is called heterozygous. When we cross two of the F1 (first filial generation) offspring resulting from the initial cross of the ~1 (parental generation) we observe two types of offspring. The F2 generation shows a ratio of approximately 3:1, three compound pinnate type-to-one webbed type. It should be remembered that phenotype ratios are theoretical. The real results may vary from the expected ratios, especially in small samples.

In this case, compound-pinnate leaf is dominant over webbed leaf, so whenever the genes w and W are combined, the dominant trait W will be expressed in the phenotype. In the F2 generation only 25% of the offspring are homozygous for W so only 25% are fixed for W. The w trait is only expressed in the F2 generation and only when two w genes are combined to form a double-recessive, fixing the recessive trait in 25% of the offspring. If compound-pinnate showed incomplete dominance over webbed, the genotypes in this example would remain the same, but the phenotypes in the F1 generation would all be intermediate types resembling both parents and the F2 phenotype ratio would be 1 compound-pinnate :2 intermediate :1 webbed.

The explanation for the predictable ratios of offspring is simple and brings us to Mendel's first law, the first of the basic rules of heredity:

> I. Each of the genes in a related pair segregate from each other during gamete formation.
>
> A common technique used to deduce the genotype of the parents is the back-cross. This is done by crossing one of the F1 progeny back to one of the true-breeding P1 parents. If the resulting ratio of phenotypes is 1:1 (one heterozygous to one homozygous) it proves that the parents were indeed homozygous dominant WW and homozygous-recessive ww.
>
> The 1:1 ratio observed when back-crossing F1 to P1 and the 1:2:1 ratio observed in F1 to F1 crosses are the two basic Mendelian ratios for the inheritance of one character controlled by one pair of genes. The astute breeder uses these ratios to determine the genotype of the parental plants and the relevance of genotype to further breeding.
>
> This simple example may be extended to include the inheritance of two or more unrelated pairs of genes at a time. For instance we might consider the simultaneous inheritance of the gene pairs T

(tall)/t (short) and M (early maturation)/m (late maturation). This is termed a polyhybrid instead of monohybrid cross. Mendel's second law allows us to predict the outcome of polyhybrid crosses also:

II. Unrelated pairs of genes are inherited independently of each other.

If complete dominance is assumed for both pairs of genes, then the 16 possible F2 genotype combinations will form 4 F2 phenotypes in a 9:3:3:1 ratio, the most frequent of which is the double-dominant tall/early condition. In complete dominance for both gene pairs would result in 9 F2 phenotypes in a 1:2:1:2:4:2:1:2:1 ratio, directly reflecting the genotype ratio. A mixed dominance condition would result in 6 F2 phenotypes in a 6:3:3:2:1:1 ratio. Thus, we see that a cross involving two independently assorting pairs of genes results in a 9:3:3:1 Mendelian phenotype ratio only if dominance is complete. This ratio may differ, depending on the dominance conditions present in the original gene pairs. Also, two new phenotypes, tall/late and short/early, have been created in the F2 generation; these phenotypes differ from both parents and grand parents. This phenomenon is termed recombination and explains the frequent observation that like begets like, but not exactly like.

A polyhybrid back-cross with two unrelated gene pairs exhibits a 1:1 ratio of phenotypes as in the mono-hybrid back-cross. It should be noted that despite dominance influence, an F1 back-cross with the P1 homozygous-recessive yields the homozygous-recessive phenotype short/late 25% of the time, and by the same logic, a back cross with the homozygous-dominant parent will yield the homozygous dominant phenotype tall/early 25% of the time. Again, the back-cross proves invaluable in determining the F1 and P1 genotypes. Since all four phenotypes of the back-cross progeny contain at least one each of both recessive genes or one each of both dominant genes, the back-cross phenotype is a direct representation of the four possible gametes produced by the F1 hybrid.

So far we have discussed inheritance of traits con trolled by discrete pairs of unrelated genes. Gene inter action is the control of a trait by two or more gene pairs. In this case genotype ratios will remain the same but phenotype ratios may be altered. Consider a hypothetical example where 2 dominant gene pairs Pp and Cc control late-season anthocyanin pigmentation (purple color) in Cannabis. If P is present alone, only the leaves of the plant (under the proper environmental stimulus) will exhibit accumulated anthocyanin pigment and turn a purple color. If C is present alone,

the plant will remain green through out its life cycle despite environmental conditions. If both are present, however, the calyxes of the plant will also exhibit accumulated anthocyanin and turn purple as the leaves do. Let us assume for now that this may be a desirable trait in Cannabis flowers. What breeding techniques can be used to produce this trait?

First, two homozygous true-breeding ~1 types are crossed and the phenotype ratio of the F1 offspring is observed.

The phenotypes of the F2 progeny show a slightly altered phenotype ratio of 9:3:4 instead of the expected 9:3:3:1 for independently assorting traits. If P and C must both be present for any anthocyanin pigmentation in leaves or calyxes, then an even more distorted phenotype ratio of 9:7 will appear.

Two gene pairs may interact in varying ways to pro duce varying phenotype ratios. Suddenly, the simple laws of inheritance have become more complex, but the data may still be interpreted.

Summary of Essential Points of Breeding

1 - The genotypes of plants are controlled by genes which are passed on unchanged from generation to generation.

2 - Genes occur in pairs, one from the gamete of the staminate parent and one from the gamete of the pistillate parent.

3 - When the members of a gene pair differ in their effect upon phenotype, the plant is termed hybrid or heterozygous.

4 - When the members of a pair of genes are equal in their effect upon phenotype, then they are termed true-breeding or homozygous.

5 - Pairs of genes controlling different phenotypic traits are (usually) inherited independently.

6 - Dominance relations and gene interaction can alter the phenotypic ratios of the F1, F2, and subsequent generations.

Polyploidy

Polyploidy is the condition of multiple sets of chromosomes within one cell. Cannabis has 20 chromosomes in the vegetative diploid (2n) condition. Triploid (3n) and tetraploid (4n) individuals have three or four sets of chromosomes and are termed polyploids. It is believed that the haploid condition of 10 chromosomes was likely derived by reduction from a higher (polyploid) ancestral number (Lewis, W. H. 1980). Polyploidy has not been shown to occur naturally in Cannabis; however, it may be induced artificially with colchicine treatments. Colchicine is a poisonous compound extracted from the roots of certain Colchicum species; it inhibits chromosome segregation to daughter cells and cell wall formation, resulting in larger than average daughter cells with multiple chromosome sets. The studies of H. E. Warmke et al. (1942-1944) seem to indicate that colchicine raised drug levels in Cannabis. It is unfortunate that Warmke was unaware of the actual psychoactive ingredients of Cannabis and was therefore unable to extract THC. His crude acetone extract and archaic techniques of bioassay using killifish and small freshwater crustaceans are far from conclusive. He was, however, able to produce both triploid and tetraploid strains of Cannabis with up to twice the potency of dip bid strains (in their ability to kill small aquatic organisms). The aim of his research was to "produce a strain of hemp with materially reduced marijuana content" and his results indicated that polyploidy raised the potency of Cannabis without any apparent increase in fiber quality or yield.

Warmke's work with polyploids shed light on the nature of sexual determination in Cannabis. He also illustrated that potency is genetically determined by creating a lower potency strain of hemp through selective breeding with low potency parents.

More recent research by A. I. Zhatov (1979) with fiber Cannabis showed that some economically valuable traits such as fiber quantity may be improved through polyploidy. Polyploids require more water and are usually more sensitive to changes in environment. Vegetative growth cycles are extended by up to 30-40% in polyploids. An extended vegetative period could delay the flowering of polyploid drug strains and interfere with the formation of floral clusters. It would be difficult to determine if cannabinoid levels had been raised by polyploidy if polyploid plants were not able to mature fully in the favorable part of the season when cannabinoid production is promoted by plentiful light and warm temperatures. Greenhouses and artificial lighting can be used to extend the season and test polyploid strains.

The height of tetraploid (4n) Cannabis in these experiments often exceeded the height of the original diploid plants by 25-30%. Tetraploids were intensely colored, with dark green leaves and stems and a well developed gross phenotype. Increased height and vigorous growth, as a rule, vanish in subsequent generations. Tetraploid plants often revert back to the diploid

condition, making it difficult to support tetraploid populations. Frequent tests are performed to determine if ploidy is changing.

Triploid (3n) strains were formed with great difficulty by crossing artificially created tetraploids (4n) with dip bids (2n). Triploids proved to be inferior to both diploids and tetraploids in many cases.

De Pasquale et al. (1979) conducted experiments with Cannabis which was treated with 0.25% and 0.50% solutions of colchicine at the primary meristem seven days after generation. Treated plants were slightly taller and possessed slightly larger leaves than the controls, Anomalies in leaf growth occurred in 20% and 39%, respectively, of the surviving treated plants. In the first group (0.25%) cannabinoid levels were highest in the plants without anomalies, and in the second group (0.50%) cannabinoid levels were highest in plants with anomalies, Overall, treated plants showed a 166-250% increase in THC with respect to controls and a decrease of CBD (30-33%) and CBN (39-65%). CBD (cannabidiol) and CBN (cannabinol) are cannabinoids involved in the biosynthesis and degradation of THC. THC levels in the control plants were very low (less than 1%). Possibly colchicine or the resulting polyploidy interferes with cannabinoid biogenesis to favor THC. In treated plants with deformed leaf lamina, 90% of the cells are tetraploid (4n 40) and 10% diploid (2n 20). In treated plants without deformed lamina a few cells are tetraploid and the remainder are triploid or diploid.

The transformation of diploid plants to the tetraploid level inevitably results in the formation of a few plants with an unbalanced set of chromosomes (2n + 1, 2n - 1, etc.). These plants are called aneuploids. Aneuploids are inferior to polyploids in every economic respect. Aneuploid Cannabis is characterized by extremely small seeds. The weight of 1,000 seeds ranges from 7 to 9 grams (1/4 to 1/3 ounce). Under natural conditions diploid plants do not have such small seeds and average 14-19 grams (1/2-2/3 ounce) per 1,000 (Zhatov 1979).

Once again, little emphasis has been placed on the relationship between flower or resin production and polyploidy. Further research to determine the effect of polyploidy on these and other economically valuable traits of Cannabis is needed.

Colchicine is sold by laboratory supply houses, and breeders have used it to induce polyploidy in Cannabis. However, colchicine is poisonous, so special care is exercised by the breeder in any use of it. Many clandestine cultivators have started polyploid strains with colchicine. Except for changes in leaf shape and phyllotaxy, no out standing characteristics have developed in these strains and potency seems unaffected. However, none of the strains have been examined to determine if they are actually polyploid or if they were merely treated with colchicine to no effect. Seed treatment is the most effective and safest way to apply colchicine. * In this way, the entire plant growing from a colchicine-treated

seed could be polyploid and if any colchicine exists at the end of the growing season the amount would be infinitesimal. Colchicine is nearly always lethal to Cannabis seeds, and in the treatment there is a very fine line between polyploidy and death. In other words, if 100 viable seeds are treated with colchicine and 40 of them germinate it is unlikely that the treatment induced polyploidy in any of the survivors. On the other hand, if 1,000 viable treated seeds give rise to 3 seedlings, the chances are better that they are polyploid since the treatment killed all of the seeds but those three. It is still necessary to determine if the offspring are actually polyploid by microscopic examination.

The work of Menzel (1964) presents us with a crude map of the chromosomes of Cannabis, Chromosomes 2-6 and 9 are distinguished by the length of each arm. Chromosome 1 is distinguished by a large knob on one end and a dark chromomere 1 micron from the knob. Chromosome 7 is extremely short and dense, and chromosome 8 is assumed to be the sex chromosome. In the future, chromosome *The word "safest" is used here as a relative term. Coichicine has received recent media attention as a dangerous poison and while these accounts are probably a bit too lurid, the real dangers of exposure to coichicine have not been fully researched. The possibility of bodily harm exists and this is multiplied when breeders inexperienced in handling toxins use colchicine. Seed treatment might be safer than spraying a grown plant but the safest method of all is to not use colchicine. mapping will enable us to picture the location of the genes influencing the phenotype of Cannabis. This will enable geneticists to determine and manipulate the important characteristics contained in the gene pool. For each trait the number of genes in control will be known, which chromosomes carry them, and where they are located along those chromosomes.

Breeding

All of the Cannabis grown in North America today originated in foreign lands. The diligence of our ancestors in their collection and sowing of seeds from superior plants, together with the forces of natural selection, have worked to create native strains with localized characteristics of resistance to pests, diseases, and weather conditions. In other words, they are adapted to particular niches in the ecosystem. This genetic diversity is nature's way of protecting a species. There is hardly a plant more flexible than Cannabis. As climate, diseases, and pests change, the strain evolves and selects new defenses, programmed into the genetic orders contained in each generation of seeds. Through the importation in recent times of fiber and drug Cannabis, a vast pool of genetic material has appeared in North America. Original fiber strains have escaped and become acclimatized (adapted to the environment), while domestic drug strains (from imported seeds) have, unfortunately, hybridized and acclimatized randomly, until many of the fine gene combinations of imported Cannabis have been lost.

Changes in agricultural techniques brought on by technological pressure, greed, and full-scale eradication programs have altered the selective pressures

influencing Cannabis genetics. Large shipments of inferior Cannabis containing poorly selected seeds are appearing in North America and elsewhere, the result of attempts by growers and smugglers to supply an ever increasing market for marijuana. Older varieties of Cannabis, associated with long standing cultural patterns, may contain genes not found in the newer commercial varieties. As these older varieties and their corresponding cultures become extinct, this genetic information could be lost forever. The increasing popularity of Cannabis and the requirements of agricultural technology will call for uniform hybrid races that are likely to displace primitive populations worldwide.

Limitation of genetic diversity is certain to result from concerted inbreeding for uniformity. Should inbred Cannabis be attacked by some previously unknown pest or disease, this genetic uniformity could prove disastrous due to potentially resistant diverse genotypes having been dropped from the population. If this genetic complement of resistance cannot be reclaimed from primitive parental material, resistance cannot be introduced into the ravaged population. There may also be currently unrecognized favorable traits which could be irretrievably dropped from the Cannabis gene pool. Human intervention can create new phenotypes by selecting and recombining existing genetic variety, but only nature can create variety in the gene pool itself, through the slow process of random mutation.

This does not mean that importation of seed and selective hybridization are always detrimental. Indeed these principles are often the key to crop improvement, but only when applied knowledgeably and cautiously. The rapid search for improvements must not jeopardize the pool of original genetic information on which adaptation relies. At this time, the future of Cannabis lies in government and clandestine collections. These collections are often inadequate, poorly selected and badly maintained. Indeed, the United Nations Cannabis collection used as the primary seed stock for worldwide governmental research is depleted and spoiled.

Several steps must be taken to preserve our vanishing genetic resources, and action must be immediate:

- Seeds and pollen should be collected directly from reliable and knowledgeable sources. Government seizures and smuggled shipments are seldom reliable seed sources. The characteristics of both parents must be known; consequently, mixed bales of randomly pollinated marijuana are not suitable seed sources, even if the exact origin of the sample is certain. Direct contact should be made with the farmer-breeder responsible for carrying on the breeding traditions that have produced the sample. Accurate records of every possible parameter of growth must be kept with carefully stored triplicate sets of seeds.
- Since Cannabis seeds do not remain viable forever, even under the best storage conditions, seed samples should he replenished every third year.

Collections should be planted in conditions as similar as possible to their original niche and allowed to reproduce freely to minimize natural and artificial selection of genes and ensure the preservation of the entire gene pool. Half of the original seed collection should be retained until the viability of further generations is confirmed, and to provide parental material for comparison and back-crossing. Phenotypic data about these subsequent generations should be carefully recorded to aid in understanding the genotypes contained in the collection. Favorable traits of each strain should be characterized and catalogued.

- It is possible that in the future, Cannabis cultivation for resale, or even personal use, may be legal but only for approved, patented strains. Special caution would be needed to preserve variety in the gene pool should the patenting of Cannabis strains become a reality.
- Favorable traits must be carefully integrated into existing strains.

The task outlined above is not an easy one, given the current legal restrictions on the collection of Cannabis seed. In spite of this, the conscientious cultivator is making a contribution toward preserving and improving the genetics of this interesting plant.

Even if a grower has no desire to attempt crop improvement, successful strains have to be protected so they do not degenerate and can be reproduced if lost. Left to the selective pressures of an introduced environment, most drug strains will degenerate and lose potency as they acclimatize to the new conditions. Let me cite an example of a typical grower with good intentions.

A grower in northern latitudes selected an ideal spot to grow a crop and prepared the soil well. Seeds were selected from the best floral clusters of several strains avail able over the past few years, both imported and domestic. Nearly all of the staminate plants were removed as they matured and a nearly seedless crop of beautiful plants resulted. After careful consideration, the few seeds from accidental pollination of the best flowers were kept for the following season, These seeds produced even bigger and better plants than the year before and seed collection was performed as before. The third season, most of the plants were not as large or desirable as the second season, but there were many good individuals. Seed collection and cultivation the fourth season resulted in plants inferior even to the first crop, and this trend continued year after year. What went wrong? The grower collected seed from the best plants each year and grew them under the same conditions. The crop improved the first year. Why did the strain degenerate?

This example illustrates the unconscious selection for undesirable traits. The hypothetical cultivator began well by selecting the best seeds available and growing them properly. The seeds selected for the second season resulted from random hybrid pollinations by early-flowering or overlooked staminate plants and by hermaphrodite pistil late plants. Many of these random pollen-parents may be

undesirable for breeding since they may pass on tendencies toward premature maturation, retarded maturation, or hermaphrodism. However, the collected hybrid seeds pro duce, on the average, larger and more desirable offspring than the first season. This condition is called hybrid vigor and results from the hybrid crossing of two diverse gene pools. The tendency is for many of the dominant characteristics from both parents to be transmitted to the F1 off spring, resulting in particularly large and vigorous plants. This increased vigor due to recombination of dominant genes often raises the cannabinoid level of the F1 offspring, but hybridization also opens up the possibility that undesirable (usually recessive) genes may form pairs and express their characteristics in the F2 offspring. Hybrid vigor may also mask inferior qualities due to abnormally rapid growth. During the second season, random pollinations again accounted for a few seeds and these were collected. This selection draws on a huge gene pool and the possible F2 combinations are tremendous. By the third season the gene pool is tending toward early-maturing plants that are acclimatized to their new conditions instead of the drug-producing conditions of their native environment. These acclimatized members of the third crop have a higher chance of maturing viable seeds than the parental types, and random pollinations will again increase the numbers of acclimatized individuals, and thereby increase the chance that undesirable characteristics associated with acclimatization will be transmitted to the next F2 generation. This effect is compounded from generation to generation and finally results in a fully acclimatized weed strain of little drug value.

With some care the breeder can avoid these hidden dangers of unconscious selection. Definite goals are vital to progress in breeding Cannabis. What qualities are desired in a strain that it does not already exhibit? What characteristics does a strain exhibit that are unfavorable and should be bred out? Answers to these questions suggest goals for breeding. In addition to a basic knowledge of Cannabis botany, propagation, and genetics, the successful breeder also becomes aware of the most minute differences and similarities in phenotype. A sensitive rapport is established between breeder and plants and at the same time strict guidelines are followed. A simplified explanation of the time-tested principles of plant breeding shows how this works in practice.

Selection is the first and most important step in the breeding of any plant. The work of the great breeder and plant wizard Luther Burbank stands as a beacon to breeders of exotic strains. His success in improving hundreds of flower, fruit, and vegetable crops was the result of his meticulous selection of parents from hundreds of thou sands of seedlings and adults from the world over.

Bear in mind that in the production of any new plant, selection plays the all-important part. First, one must get clearly in mind the kind of plant he wants, then breed and select to that end, always choosing through a series of years the plants which are approaching nearest the ideal, and rejecting all others.

- Luther Burbank (in James, 1964)

Proper selection of prospective parents is only possible if the breeder is familiar with the variable characteristics of Cannabis that may be genetically controlled, has a way to accurately measure these variations, and has established goals for improving these characteristics by selective breeding. A detailed list of variable traits of Cannabis, including parameters of variation for each trait and comments pertaining to selective breeding for or against it, are found at the end of this chapter. By selecting against unfavorable traits while selecting for favorable ones, the unconscious breeding of poor strains is avoided.

The most important part of Burbank's message on selection tells breeders to choose the plants "which are approaching nearest the ideal," and REJECT ALL OTHERS! Random pollinations do not allow the control needed to reject the undesirable parents. Any staminate plant that survives detection and roguing (removal from the population), or any stray staminate branch on a pistillate her maphrodite may become a pollen parent for the next generation. Pollination must be controlled so that only the pollen- and seed-parents that have been carefully selected for favorable traits will give rise to the next generation.

Selection is greatly improved if one has a large sample to choose from! The best plant picked from a group of 10 has far less chance of being significantly different from its fellow seedlings than the best plant selected from a sample of 100,000. Burbank often made his initial selections of parents from samples of up to 500,000 seedlings. Difficulties arise for many breeders because they lack the space to keep enough examples of each strain to allow a significant selection. A Cannabis breeder's goals are restricted by the amount of space available. Formulating a well defined goal lowers the number of individuals needed to perform effective crosses. Another technique used by breeders since the time of Burbank is to make early selections. Seedling plants take up much less space than adults. Thousands of seeds can be germinated in a flat. A flat takes up the same space as a hundred 10-centimeter (4-inch) sprouts or six-teen 30-centimeter (12-inch) seedlings or one 60-centimeter (24-inch) juvenile. An adult plant can easily take up as much space as a hundred flats. Simple arithmetic shows that as many as 10,000 sprouts can be screened in the space required by each mature plant, provided enough seeds are available. Seeds of rare strains are quite valuable and exotic; however, careful selection applied to thousands of individuals, even of such common strains as those from Colombia or Mexico, may produce better offspring than plants from a rare strain where there is little or no opportunity for selection after germination. This does not mean that rare strains are not valuable, but careful selection is even more important to successful breeding. The random pollinations that produce the seeds in most imported marijuana assure a hybrid condition which results in great seed ling diversity. Distinctive plants are not hard to discover if the seedling sample is large enough.

Traits considered desirable when breeding Cannabis often involve the yield and quality of the final product, but these characteristics can only be accurately

measured after the plant has been harvested and long after it is possible to select or breed it. Early seedling selection, therefore, only works for the most basic traits. These are selected first, and later selections focus on the most desirable characteristics exhibited by juvenile or adult plants. Early traits often give clues to mature phenotypic expression, and criteria for effective early seedling selection are easy to establish. As an example, particularly tall and thin seedlings might prove to be good parents for pulp or fiber production, while seed lings of short internode length and compound branching may be more suitable for flower production. However, many important traits to be selected for in Cannabis floral clusters cannot be judged until long after the parents are gone, so many crosses are made early and selection of seeds made at a later date.

Hybridization is the process of mixing differing gene pools to produce offspring of great genetic variation from which distinctive individuals can be selected. The wind performs random hybridization in nature. Under cultivation, breeders take over to produce specific, controlled hybrids. This process is also known as cross-pollination, cross-fertilization, or simply crossing. If seeds result, they will produce hybrid offspring exhibiting some characteristics from each parent.

Large amounts of hybrid seed are most easily produced by planting two strains side by side, removing the staininate plants of the seed strain, and allowing nature to take its course. Pollen- or seed-sterile strains could be developed for the production of large amounts of hybrid seed without the labor of thinning; however, genes for sterility are rare. It is important to remember that parental weak nesses are transmitted to offspring as well as strengths. Because of this, the most vigorous, healthy plants are al ways used for hybrid crosses.

Also, sports (plants or parts of plants carrying and expressing spontaneous mutations) most easily transmit mutant genes to the offspring if they are used as pollen parents. If the parents represent diverse gene pools, hybrid vigor results, because dominant genes tend to carry valuable traits and the differing dominant genes inherited from each parent mask recessive traits inherited from the other. This gives rise to particularly large, healthy individuals. To increase hybrid vigor in offspring, parents of different geo graphic origins are selected since they will probably represent more diverse gene pools.

Occasionally hybrid offspring will prove inferior to both parents, but the first generation may still contain recessive genes for a favorable characteristic seen in a parent if the parent was homozygous for that trait. First generation (F1) hybrids are therefore inbred to allow recessive genes to recombine and express the desired parental trait. Many breeders stop with the first cross and never realize the genetic potential of their strain. They fail to produce an F2 generation by crossing or self-pollinating F1 offspring. Since most domestic Cannabis strains are F1 hybrids for many characteristics, great diversity and recessive recombination can result from inbreeding domestic hybrid strains. In this way the breeding of the F1 hybrids has already been accomplished, and a year is saved

by going directly to F2 hybrids. These F2 hybrids are more likely to express recessive parental traits. From the F2 hybrid generation selections can be made for parents which are used to start new true-breeding strains. Indeed, F2 hybrids might appear with more extreme characteristics than either of the P~ parents. (For example, P1 high-THC X P1 low-THC yields F1 hybrids of intermediate THC content. Selfing the F1 yields F2 hybrids, of both P1 [high and low THC] phenotypes, inter mediate F1 phenotypes, and extra-high THC as well as extra-low THC phenotypes.)

Also, as a result of gene recombination, F1 hybrids are not true-breeding and must be reproduced from the original parental strains. When breeders create hybrids they try to produce enough seeds to last for several successive years of cultivation, After initial field tests, undesirable hybrid seeds are destroyed and desirable hybrid seeds stored for later use. If hybrids are to be reproduced, a clone is saved from each parental plant to preserve original parental genes.

Back-crossing is another technique used to produce offspring with reinforced parental characteristics. In this case, a cross is made between one of the F~ or subsequent offspring and either of the parents expressing the desired trait. Once again this provides a chance for recombination and possible expression of the selected parental trait. Back-crossing is a valuable way of producing new strains, but it is often difficult because Cannabis is an annual, so special care is taken to save parental stock for back-crossing the following year. Indoor lighting or greenhouses can be used to protect breeding stock from winter weather. In tropical areas plants may live outside all year. In addition to saving particular parents, a successful breeder always saves many seeds from the original P1 group that produced the valuable characteristic so that other P1 plants also exhibiting the characteristic can be grown and selected for back-crossing at a later time.

Several types of breeding are summarized as follows:

1 - Crossing two varieties having outstanding qualities (hybridization).

2 - Crossing individuals from the F1 generation or selfing F1 individuals to realize the possibilities of the original cross (differentiation).

3 - Back crossing to establish original parental types.

4 - Crossing two similar true-breeding (homozygous) varieties to preserve a mutual trait and restore vigor.

It should be noted that a hybrid plant is not usually hybrid for all characteristics nor does a true-breeding strain breed true for all characteristics. When discussing crosses, we are talking about the inheritance of one or a few traits

only. The strain may be true-breeding for only a few traits, hybrid for the rest. Monohybrid crosses involve one trait, dihybrid crosses involve two traits, and so forth. Plants have certain limits of growth, and breeding can only pro duce a plant that is an expression of some gene already present in the total gene pool. Nothing is actually created by breeding; it is merely the recombination of existing genes into new genotypes. But the possibilities of recombination are nearly limitless.

The most common use of hybridization is to cross two outstanding varieties. Hybrids can be produced by crossing selected individuals from different high-potency strains of different origins, such as Thailand and Mexico. These two parents may share only the characteristic of high psycho activity and differ in nearly every other respect. From this great exchange of genes many phenotypes may appear in the F2 generation. From these offspring the breeder selects individuals that express the best characteristics of the parents. As an example, consider some of the offspring from the P1 (parental) cross: Mexico X Thailand. In this case, genes for high drug content are selected from both parents while other desirable characteristics can be selected from either one. Genes for large stature and early maturation are selected from the Mexican seed-parent, and genes for large calyx size and sweet floral aroma are selected from the Thai pollen parent. Many of the F1 offspring exhibit several of the desired characteristics. To further promote gene segregation, the plants most nearly approaching the ideal are crossed among themselves. The F2 generation is a great source of variation and recessive expression. In the F2 generation there are several individuals out of many that exhibit all five of the selected characteristics. Now the process of inbreeding begins, using the desirable F2 parents.

If possible, two or more separate lines are started, never allowing them to interbreed. In this case one accept able staminate plant is selected along with two pistillate plants (or vice versa). Crosses between the pollen parent and the two seed parents result in two lines of inheritance with slightly differing genetics, but each expressing the desired characteristics. Each generation will produce new, more acceptable combinations.

If two inbred strains are crossed, F1 hybrids will be less variable than if two hybrid strains are crossed. This comes from limiting the diversity of the gene pools in the two strains to be hybridized through previous inbreeding. Further independent selection and inbreeding of the best plants for several generations will establish two strains which are true-breeding for all the originally selected traits. This means that all the offspring from any parents in the strain will give rise to seedlings which all exhibit the selected traits. Successive inbreeding may by this time have resulted in steady decline in the vigor of the strain.

When lack of vigor interferes with selecting phenotypes for size and hardiness, the two separately selected strains can then be interbred to recombine

nonselected genes and restore vigor. This will probably not interfere with breeding for the selected traits unless two different gene systems control the same trait in the two separate lines, and this is highly unlikely. Now the breeder has produced a hybrid strain that breeds true for large size, early maturation, large sweet-smelling calyxes, and high THC level. The goal has been reached!

Wind pollination and dioecious sexuality favor a heterozygous gene pool in Cannabis. Through Anbreeding, hybrids are adapted from a heterozygous gene pool to a homozygous gene pool, providing the genetic stability needed to create true-breeding strains. Establishing pure strains enables the breeder to make hybrid crosses with a better chance of predicting the outcome. Hybrids can be created that are not reproducible in the F2 generation. Commercial strains of seeds could be developed that would have to be purchased each year, because the F1 hybrids of two pure-bred lines do not breed true. Thus, a seed breeder can protect the investment in the results of breeding, since it would be nearly impossible to reproduce the parents from F2 seeds.

At this time it seems unlikely that a plant patent would be awarded for a pure-breeding strain of drug Cannabis. In the future, however, with the legalization of cultivation, it is a certainty that corporations with the time, space, and money to produce pure and hybrid strains of Cannabis will apply for patents. It may be legal to grow only certain patented strains produced by large seed companies. Will this be how government and industry combine to control the quality and quantity of "drug" Cannabis?

Acclimatization

Much of the breeding effort of North American cultivators is concerned with acclimatizing high-THC strains of equatorial origin to the climate of their growing area while preserving potency. Late-maturing, slow, and irregularly flowering strains like those of Thailand have difficulty maturing in many parts of North America. Even in a green house, it may not be possible to mature plants to their full native potential.

To develop an early-maturing and rapidly flowering 8train, a breeder may hybridize as in the previous example. However, if it is important to preserve unique imported genetics, hybridizing may be inadvisable. Alternatively, a pure cross is made between two or more Thai plants that most closely approach the ideal in blooming early. At this point the breeder may ignore many other traits and aim at breeding an earlier-maturing variety of a pure Thai strain. This strain may still mature considerably later than is ideal for the particular location unless selective pressure is exerted. If further crosses are made with several individuals that satisfy other criteria such as high THC content, these may be used to develop another pure Thai strain of high THC content. After these true-breeding lines have been established, a dihybrid pure cross can be made in an attempt to

produce an F1 generation containing early-maturing, high-THC strains of pure Thai genetics, in other words, an acclimatized drug strain.

Crosses made without a clear goal in mind lead to strains that acclimatize while losing many favorable characteristics. A successful breeder is careful not to overlook a characteristic that may prove useful. It is imperative that original imported Cannabis genetics be preserved intact to protect the species from loss of genetic variety through excessive hybridization. A currently unrecognized gene may be responsible for controlling resistance to a pest or disease, and it may only be possible to breed for this gene by back-crossing existing strains to original parental gene pools.

> Once pure breeding lines have been established, plant breeders classify and statistically analyze the offspring to determine the patterns of inheritance for that trait. This is the system used by Gregor Mendel to formulate the basic laws of inheritance and aid the modern breeder in predicting the outcome of crosses,
>
> 1 - Two pure lines of Cannabis that differ in a particular trait are located.
>
> 2 - These two pure-breeding lines are crossed to pro duce an F1 generation.
>
> 3 - The F1 generation is inbred.
>
> 4 - The offspring of the F1 and F2 generations are classified with regard to the trait being studied.
>
> 5 - The results are analyzed statistically.
>
> 6 - The results are compared to known patterns of inheritance so the nature of the genes being selected for can be characterized.

Fixing Traits

Fixing traits (producing homozygous offspring) in Cannabis strains is more difficult than it is in many other flowering plants. With monoecious strains or hermaphrodites it is possible to fix traits by self-pollinating an individual exhibiting favorable traits. In this case one plant acts as both mother and father. However, most strains of Cannabis are dioecious, and unless hermaphroditic reactions can be induced, another parent exhibiting the trait is required to fix the trait. If this is not possible, the unique individual may be crossed with a plant not exhibiting the

trait, inbred in the F1 generation, and selections of parents exhibiting the favorable trait made from the F2 generation, but this is very difficult.

If a trait is needed for development of a dioecious strain it might first be discovered in a monoecious strain and then fixed through selfing and selecting homozygous offspring. Dioecious individuals can then be selected from the monoecious population and these individuals crossed to breed out monoecism in subsequent generations.

Galoch (1978) indicated that gibberellic acid (GA3) promoted stamen production while indoleacetic acid (IAA), ethrel, and kinetin promoted pistil production in prefloral dioecious Cannabis. Sex alteration has several useful applications. Most importantly, if only one parent expressing a desirable trait can be found, it is difficult to perform a cross unless it happens to be a hermaphrodite plant. Hormones might be used to change the sex of a cutting from the desirable plant, and this cutting used to mate with it. This is most easily accomplished by changing a pistillate cutting to a staminate (pollen) parent, using a spray of 100 ppm gibberellic acid in water each day for five consecutive days. Within two weeks staminate flowers may appear. Pollen can then be collected for selfing with the original pistillate parent. Offspring from the cross should also be mostly pistillate since the breeder is selfing for pistillate sexuality. Staminate parents reversed to pistillate floral production make inferior seed-parents since few pistillate flowers and seeds are formed.

If entire crops could be manipulated early in life to produce all pistillate or staminate plants, seed production and seedless drug Cannabis production would be greatly facilitated.

Sex reversal for breeding can also be accomplished by mutilation and by photoperiod alteration. A well-rooted, flourishing cutting from the parent plant is pruned back to 25% of its original size and stripped of all its remaining flowers. New growth will appear within a few days, and several flowers of reversed sexual type often appear. Flowers of the unwanted sex are removed until the cutting is needed for fertilization. Extremely short light cycles (6-8 hour photoperiod) can also cause sex reversal. How ever, this process takes longer and is much more difficult to perform in the field.

Genotype and Phenotype Ratios

It must be remembered, in attempting to fix favorable characteristics, that a monohybrid cross gives rise to four possible recombinant genotypes, a dihybrid cross gives rise to 16 possible recombinant genotypes, and so forth.

Phenotype and genotype ratios are probabilistic. If recessive genes are desired for three traits it is not effective to raise only 64 offspring and count on getting one homozygous recessive individual. To increase the probability of success it is

better to raise hundreds of offspring, choosing only the best homozygous recessive individuals as future parents. All laws of inheritance are based on chance and offspring may not approach predicted ratios until many more have been phenotypically characterized and grouped than the theoretical minimums.

The genotype of each individual is expressed by a mosaic of thousands of subtle overlapping traits. It is the sum total of these traits that determines the general phenotype of an individual. It is often difficult to determine if the characteristic being selected is one trait or the blending of several traits and whether these traits are controlled by one or several pairs of genes. It often makes little difference that a breeder does not have plants that are proven to breed true. Breeding goals can still be established. The selfing of F1 hybrids will often give rise to the variation needed in the F2 generation for selecting parents for subsequent generations, even if the characteristics of the original parents of the F1 hybrid are not known. It is in the following generations that fixed characteristics appear and the breeding of pure strains can begin. By selecting and crossing individuals that most nearly approach the ideal described by the breeding goals, the variety can be continuously improved even if the exact patterns of inheritance are never deter mined. Complementary traits are eventually combined into one line whose seeds reproduce the favorable parental traits. Inbreeding strains also allows weak recessive traits to express themselves and these abnormalities must be diligently removed from the breeding population. After five or six generations, strains become amazingly uniform. Vigor is occasionally restored by crossing with other lines or by backcrossing.

Parental plants are selected which most nearly approach the ideal. If a desirable trait is not expressed by the parent, it is much less likely to appear in the offspring. It is imperative that desirable characteristics be hereditary and not primarily the result of environment and cultivation. Acquired traits are not hereditary and cannot be made hereditary. Breeding for as few traits as possible at one time greatly increases the chance of success. In addition to the specific traits chosen as the aims of breeding, parents are selected which possess other generally desirable traits such as vigor and size. Determinations of dominance and recessiveness can only be made by observing the outcome of many crosses, although wild traits often tend to be dominant. This is one of the keys to adaptive survival. However, all the possible combinations will appear in the F2 generation if it is large enough, regardless of dominance.

Now, after further simplifying this wonderful system of inheritance, there are additional exceptions to the rules which must be explored. In some cases, a pair of genes may control a trait but a second or third pair of genes is needed to express this trait. This is known as gene inter action. No particular genetic attribute in which we may be interested is totally isolated from other genes and the effects of environment. Genes are occasionally transferred in groups instead of assorting independently. This is known as gene linkage, These genes are spaced along the same chromosome and may or may not control the same trait.

The result of linkage might be that one trait cannot be inherited without another. At times, traits are associated with the X and Y sex chromosomes and they may be limited to expression in only one sex (sex linkage). Crossing over also interferes with the analysis of crosses. Crossing over is the exchanging of entire pieces of genetic material between two chromosomes. This can result in two genes that are normally linked appearing on separate chromosomes where they will be independently inherited. All of these processes can cause crosses to deviate from the expected Mendelian outcome. Chance is a major factor in breeding Cannabis, or any introduced plant, and the more crosses a breeder attempts the higher are the chances of success.

Variate, isolate, intermate, evaluate, multiplicate, and disseminate are the key words in plant improvement. A plant breeder begins by producing or collecting various prospective parents from which the most desirable ones are selected and isolated. Intermating of the select parents results in offspring which must be evaluated for favorable characteristics. If evaluation indicates that the offspring are not improved, then the process is repeated. Improved off spring are multiplied and disseminated for commercial use. Further evaluation in the field is necessary to check for uniformity and to choose parents for further intermating. This cyclic approach provides a balanced system of plant improvement.

The basic nature of Cannabis makes it challenging to

breed. Wind pollination and dioecious sexuality, which

account for the great adaptability in Cannabis, cause many

problems in breeding, but none of these are insurmountable. Developing a knowledge and feel for the plant is more important than memorizing Mendelian ratios. The words of the great Luther Burbank say it well, "Heredity is indelibly fixed by repetition."

The first set of traits concerns Cannabis plants as a whole while the remainder concern the qualities of seedlings, leaves, fibers, and flowers. Finally a list of various Cannabis strains is provided along with specific characteristics. Following this order, basic and then specific selections of favorable characteristics can be made.

List of Favorable Traits of Cannabis

in Which Variation Occurs

1. General Traits

a) Size and Yield

b) Vigor

c) Adaptability

d) Hardiness

e) Disease and Pest Resistance

f) Maturation

g) Root Production

h) Branching

i) Sex

2. Seedling Traits

3. Leaf Traits

4. Fiber Traits

5. Floral Traits

a) Shape

b) Form

c) Calyx Size

d) Color

e) Cannabinoid Level

f) Taste and Aroma

g) Persistence of Aromatic Principles and Cannabinoids

h) Trichome Type

i) Resin Quantity and Quality

j) Resin Tenacity

k) Drying and Curing Rate

l) Ease of Manicuring

m) Seed Characteristics

n) Maturation

o) Flowering

p) Ripening

q) Cannabinoid Profile

6. Gross Phenotypes of Cannabis Strains

1. General Traits

a) Size and Yield - The size of an individual Cannabis plant is determined by environmental factors such as room for root and shoot growth, adequate light and nutrients, and proper irrigation. These environmental factors influence the phenotypic image of genotype, but the genotype of the individual is responsible for overall variations in gross morphology, including size. Grown under the same conditions, particularly large and small individuals are easily spotted and selected. Many dwarf Cannabis plants have been re ported and dwarfism may be subject to genetic control, as it is in many higher plants, such as dwarf corn and citrus. Cannabis parents selected for large size tend to produce offspring of a larger average size each year. Hybrid crosses between tall (Cannabis sativa-Mexico) strains and short (Cannabis ruderalis-Russia) strains yield F1 offspring of intermediate height (Beutler and der Marderosian 1978). Hybrid vigor, however, will influence the size of offspring more than any other genetic factor. The increased size of hybrid offspring is often amazing and accounts for much of the success of Cannabis cultivators in raising large plants. It is not known whether there is a set of genes for "gigantism" in Cannabis or whether polyploid individuals really yield more than diploid due to increased chromosome count. Tetraploids tend to be taller and their water re quirements are often higher than diploids. Yield is determined by the overall

production of fiber, seed, or resin and selective breeding can be used to increase the yield of any one of these products. However, several of these traits may be closely related, and it may be impossible to breed for one without the other (gene linkage). Inbreeding of a pure strain increases yield only if high yield parents are selected. High yield plants, staminate or pistillate, are not finally selected until the plants are dried and manicured. Because of this, many of the most vigorous plants are crossed and seeds selected after harvest when the yield can be measured.

b) Vigor - Large size is often also a sign of healthy vigorous growth. A plant that begins to grow immediately will usually reach a larger size and produce a higher yield in a short growing season than a sluggish, slow-growing plant. Parents are always selected for rich green foliage and rapid, responsive growth. This will ensure that genes for certain weaknesses in overall growth and development are bred out of the population while genes for strength and vigor remain.

c) Adaptability - It is important for a plant with a wide distribution such as Cannabis to be adaptable to many different environmental conditions. Indeed, Cannabis is one of the most genotypically diverse and phenotypically plastic plants on earth; as a result it has adapted to environ mental conditions ranging from equatorial to temperate climates. Domestic agricultural circumstances also dictate that Cannabis must be grown under a great variety of conditions,

Plants to be selected for adaptability are cloned and grown in several locations. The parental stocks with the highest survival percentages can be selected as prospective parents for an adaptable strain. Adaptability is really just another term for hardiness under varying growth conditions.

d) Hardiness - The hardiness of a plant is its overall resistance to heat and frost, drought and overwatering, and so on. Plants with a particular resistance appear when adverse conditions lead to the death of the rest of a large population. The

surviving few members of the population might carry inheritable resistance to the environmental factor that destroyed the majority of the population. Breeding these survivors, subjecting the offspring to continuing stress conditions, and selecting carefully for several generations should result in a pure-breeding strain with increased resistance to drought, frost, or excessive heat.

e) Disease and Pest Resistance - In much the same way as for hardiness a strain may be bred for resistance to a certain disease, such as damping-off fungus. If flats of seedlings are infected by damping-off disease and nearly all of them die, the remaining few will have some resistance to damping-off fungus. If this resistance is inheritable, it can be passed on to subsequent generations by crossing these surviving plants. Subsequent crossing, tested by inoculating flats of seedling offspring with damping-off fungus, should yield a more resistant strain.

Resistance to pest attack works in much the same way. It is common to find stands of Cannabis where one or a few plants are infested with insects while adjacent plants are untouched. Cannabinoid and terpenoid resins are most probably responsible for repelling insect attack, and levels of these vary from plant to plant. Cannabis has evolved defenses against insect attack in the form of resin-secreting glandular trichomes, which cover the reproductive and associated vegetative structures of mature plants. Insects, finding the resin disagreeable, rarely attack mature Cannabis flowers. However, they may strip the outer leaves of the same plant because these develop fewer glandular tri chomes and protective resins than the flowers. Non-glandular cannabinoids and other compounds produced within leaf and stem tissues which possibly inhibit insect attack, may account for the varying resistance of seedlings and vegetative juvenile plants to pest infestation. With the popularity of greenhouse Cannabis cultivation, a strain is needed with increased resistance to mold, mite, aphid,- or white fly infestation. These problems are often so severe that greenhouse cultivators destroy any plants which are attacked. Molds usually reproduce by wind-borne spores, so negligence can

rapidly lead to epidemic disaster. Selection and breeding of the least infected plants should result in strains with increased resistance.

f) Maturation - Control of the maturation of Cannabis is very important no matter what the reason for growing it. If Cannabis is to be grown for fiber it is important that the maximum fiber content of the crop be reached early and that all of the individuals in the crop mature at the same time to facilitate commercial harvesting. Seed production requires the even maturation of both pollen and seed parents to ensure even setting and maturation of seeds. An uneven maturation of seeds would mean that some seeds would drop and be lost while others are still ripening. An understanding of floral maturation is the key to the production of high quality drug Cannabis. Changes in gross morphology are accompanied by changes in cannabinoid and terpenoid production and serve as visual keys to deter mining the ripeness of Cannabis flowers.

A Cannabis plant may mature either early or late, be fast or slow to flower, and ripen either evenly or sequentially.

Breeding for early or late maturation is certainly a reality; it is also possible to breed for fast or slow flowering and even or sequential ripening. In general, crosses between early-maturing plants give rise to early-maturing offspring, crosses between late-maturing plants give rise to late-maturing offspring, and crosses between late- and early-maturing plants give rise to offspring of intermediate maturation. This seems to indicate that maturation of Cannabis is not controlled by the simple dominance and recessiveness of one gene but probably results from incomplete dominance and a combination of genes for separate aspects of maturation. For instance, Sorghum maturation is controlled by four separate genes. The sum of these genes produces a certain phenotype for maturation. Al though breeders do not know the action of each specific gene, they still can breed for the total of these traits and achieve results more nearly approaching the goal of timely maturation than the parental strains.

g) Root Production - The size and shape of Cannabis root systems vary greatly. Although every embryo sends out a taproot from which lateral roots grow, the individual growth pattern and final size and shape of the roots vary considerably. Some plants send out a deep taproot, up to 1 meter (39 inches) long, which helps support the plant against winds and rain. Most Cannabis plants, however, produce a poor taproot which rarely extends more than 30 centimeters (1 foot). Lateral growth is responsible for most of the roots in Cannabis plants. These fine lateral roots offer the plant additional support but their primary function is to absorb water and nutrients from the soil. A large root system will be able to feed and support a large plant. Most lateral roots grow near the surface of the soil where there is more water, more oxygen, and more avail able nutrients. Breeding for root size and shape may prove beneficial for the production of large rain- and wind-resistant strains. Often Cannabis plants, even very large ones, have very small and sensitive root systems. Recently, certain alkaloids have been discovered in the roots of Cannabis that might have some medical value. If this proves the case, Cannabis may be cultivated and bred for high alkaloid levels in the roots to be used in the commercial production of pharmaceuticals.

As with many traits, it is difficult to make selections for root types until the parents are harvested. Because of this many crosses are made early and seeds selected later.

h) Branching - The branching pattern of a Cannabis plant is determined by the frequency of nodes along each branch and the extent of branching at each node. For examples, consider a tall, thin plant with slender limbs made up of long internodes and nodes with little branching (Oaxaca, Mexico strain). Compare this with a stout, densely branched plant with limbs of short internodes and highly branched nodes (Hindu Kush hashish strains). Different branching patterns are preferred for the different agricultural applications of fiber, flower, or resin production. Tall, thin plants with long internodes and no branching are best adapted to fiber production; a short, broad plant with short inter nodes and well

developed branching is best adapted to floral production. Branching structure is selected that will tolerate heavy rains and high winds without breaking. This is quite advantageous to outdoor growers in temperate zones with short seasons. Some breeders select tall, limber plants (Mexico) which bend in the wind; others select short, stiff plants (Hindu Kush) which resist the weight of water without bending.

i) Sex - Attempts to breed offspring of only one sexual type have led to more misunderstanding than any other facet of Cannabis genetics. The discoveries of McPhee (1925) and Schaffner (1928) showed that pure sexual type and hermaphrodite conditions are inherited and that the percentage of sexual types could be altered by crossing with certain hermaphrodites. Since then it has generally been assumed by researchers and breeders that a cross between ANY unselected hermaphrodite plant and a pistillate seed-parent should result in a population of all pistillate offspring. This is not the case. In most cases, the offspring of hermaphrodite parents tend toward hermaphrodism, which is largely unfavorable for the production of Cannabis other than fiber hemp. This is not to say that there is no tendency for hermaphrodite crosses to alter sex ratios in the offspring. The accidental release of some pollen from predominantly pistillate hermaphrodites, along with the complete eradication of nearly every staminate and staminate hermaphrodite plant may have led to a shift in sexual ratio in domestic populations of sinsemilla drug Cannabis. It is commonly observed that these strains tend toward 60% to 80% pistillate plants and a few pistillate hermaphrodites are not uncommon in these populations.

However, a cross can be made which will produce nearly all pistillate or staminate individuals. If the proper pistillate hermaphrodite plant is selected as the pollen-parent and a pure pistillate plant is selected as the seed-parent it is possible to produce an F1, and subsequent generations, of nearly all pistillate offspring. The proper pistillate hermaphrodite pollen-parent is one which has grown as a pure pistillate plant and at the end of the sea son, or under artificial environmental stress, begins to develop a very few

staminate flowers. If pollen from these few staminate flowers forming on a pistillate plant is applied to a pure pistillate seed parent, the resulting F1 generation should be almost all pistillate with only a few pistillate hermaphrodites. This will also be the case if the selected pistillate hermaphrodite pollen source is selfed and bears its own seeds. Remember that a selfed hermaphrodite gives rise to more hermaphrodites, but a selfed pistillate plant that has given rise to a limited number of staminate flowers in response to environmental stresses should give rise to nearly all pistillate offspring. The F1 offspring may have a slight tendency to produce a few staminate flowers under further environmental stress and these are used to produce F2 seed. A monoecious strain produces 95+% plants with many pistillate and staminate flowers, but a dioecious strain produces 95+% pure pistillate or staminate plants. A plant from a dioecious strain with a few inter sexual flowers is a pistillate or staminate hermaphrodite. Therefore, the difference between monoecism and her maphrodism is one of degree, determined by genetics and environment.

Crosses may also be performed to produce nearly all staminate offspring. This is accomplished by crossing a pure staminate plant with a staminate plant that has produced a few pistillate flowers due to environmental stress, or selfing the latter plant. It is readily apparent that in the wild this is not a likely possibility. Very few staminate plants live long enough to produce pistillate flowers, and when this does happen the number of seeds produced is limited to the few pistillate flowers that occur. In the case of a pistillate hermaphrodite, it may produce only a few staminate flowers, but each of these may produce thou sands of pollen grains, any one of which may fertilize one of the plentiful pistillate flowers, producing a seed. This is another reason that natural Cannabis populations tend toward predominantly pistillate and pistillate hermaphrodite plants. Artificial hermaphrodites can be produced by hormone sprays, mutilation, and altered light cycles. These should prove most useful for fixing traits and sexual type.

Drug strains are selected for strong dioecious tendencies. Some breeders select strains with a sex ratio more nearly approaching one than a strain with a high pistillate sex ratio. They believe this reduces the chances of pistillate plants turning hermaphrodite later in the season.

2. Seedling Traits

Seedling traits can be very useful in the efficient and purposeful selection of future parental stock. If accurate selection can be exercised on small seedlings, much larger populations can be grown for initial selection, as less space is required to raise small seedlings than mature plants. Whorled phyllotaxy and resistance to damping-off are two traits that may be selected just after emergence of the embryo from the soil. Early selection for vigor, hardiness, resistance, and general growth form may be made when the seedlings are from 30 to 90 centimeters (1 to 3 feet) tall. Leaf type, height, and branching are other criteria for early selection. These early-selected plants cannot be bred until they mature, but selection is the primary and most important step in plant improvement.

Whorled phyllotaxy is associated with subsequent anomalies in the growth cycle (i.e., multiple leaflets and flattened or clubbed stems). Also, most whorled plants are staminate and whorled phyllotaxy may be sex-linked.

3. Leaf Traits

Leaf traits vary greatly from strain to strain. In addition to these regularly occurring variations in leaves, there are a number of mutations and possible traits in leaf shape. It may turn out that leaf shape is correlated with other traits in Cannabis. Broad leaflets might be associated with a low calyx-to-leaf ratio and narrow leaflets might be associated with a high calyx-to-leaf ratio. If this is the case, early selection of seedlings by leaflet shape could determine the character of the flowering clusters at harvest. Both compound and webbed leaf variations seem to be hereditary, as are general leaf characteristics. A breeder may wish to develop a unique leaf shape for an ornamental strain or increase leaf yield for pulp production.

A peculiar leaf mutation was reported from an F1-Colombian plant in which two leaves on the plant, at the time of flowering, developed floral clusters of 5-10 pistil late calyxes at the intersection of the leaflet array and the petiole attachment, on the adaxial (top) side of the leaf. One of these clusters developed a partial staminate flower but fertilization was unsuccessful. It is unknown if this mutation is hereditary.

From Afghanistan, another example has been observed with several small floral clusters along the petioles of many of the large primary leaves.

4. Fiber Traits

More advanced breeding has occurred in fiber strains than any other type of Cannabis. Over the years many strains have been developed with improved maturation, in creased fiber content, and improved fiber quality as regards length, strength, and suppleness. Extensive breeding programs have been carried on in France, Italy, Russia, and the United States to develop better varieties of fiber Cannabis. Tall limbless strains that are monoecious are most desirable. Monoeciousness is favored, because in dioecious populations the staminate plants will mature first and the fibers will become brittle before the pistillate plants are ready for harvest. The fiber strains of Europe are divided into northern and southern varieties. The latter require higher temperatures and a longer vegetative period and as a result grow taller and yield more fiber.

5. Floral Traits

Many individual traits determine the floral characteristics of Cannabis This section will focus on the individual traits of pistillate floral clusters with occasional comments about similar traits in staminate floral clusters. Pistillate flowering clusters are the seed-producing organs of Cannabis; they remain on the plant and go through many changes that cannot be compared to staminate plants.

a) Shape - The basic shape of a floral cluster is determined by the internode lengths along the main floral axis and within individual floral clusters. Dense, long clusters result when internodes are short along a long floral axis and there are short internodes within

the individual compact floral clusters (Hindu Kush). Airy clusters result when a plant forms a stretched floral axis with long internodes between well-branched individual floral clusters (Thailand).

The shape of a floral cluster is also determined by the general growth habit of the plant. Among domestic Cannabis phenotypes, for instance, it is obvious that floral clusters from a creeper phenotype plant will curve upwards at the end, and floral clusters from the huge upright phenotype will have long, straight floral clusters of various shapes. Early in the winter, many strains begin to stretch and cease calyx production in preparation for rejuvenation and sub sequent vegetative growth in the spring. Staminate plants also exhibit variation in floral clusters. Some plants have tight clusters of staminate calyxes resembling inverted grapes (Hindu Kush) and others have long, hanging groups of flowers on long, exposed, leafless branches (Thailand).

b) Form - The form of a floral cluster is determined by the numbers and relative proportions of calyxes and flowers. A leafy floral cluster might be 70% leaves and have a calyx-to-leaf ratio of 1-to-4. It is obvious that strains with a high calyx-to-leaf ratio are more adapted to calyx production, and therefore, to resin production. This factor could be advantageous in characterizing plants as future parents of drug strains. At this point it must be noted that pistillate floral clusters are made up of a number of distinct parts. They include stems, occasional seeds, calyxes, inner leaves subtending calyx pairs (small, resinous, 1-3 leaflets), and outer leaves subtending entire floral clusters (larger, little resin, 3-11 leaflets). The ratios (by dry weight) of these various portions vary by strain, degree of pollination, and maturity of the floral clusters. Maturation is a reaction to environmental change, and the degree of maturity reached is subject to climatic limits as well as breeder's preference. Because of this interplay between environment and genetics in the control of floral form it is often difficult to breed Cannabis for floral characteristics. A thorough knowledge of the way a strain matures is important in separating possible inherited traits of floral clusters from acquired traits. Chapter IV,

Maturation and Harvesting of Cannabis, delves into the secrets and theories of maturation. For now, we will assume that the following traits are described from fully mature floral clusters (peak floral stage) before any decline.

c) Calyx Size - Mature calyxes range in size from 2 to 12 millimeters (1/16 to 3/8 inch) in length. Calyx size is largely dependent upon age and maturity. Calyx size of a floral cluster is best expressed as the average length of the mature viable calyxes. Calyxes are still considered viable if both pistils appear fresh and have not begun to curl or change colors. At this time, the calyx is relatively straight and has not begun to swell with resin and change shape as it will when the pistils die. It is generally agreed that the production of large calyxes is often as important in deter mining the psychoactivity of a strain as the quantity of calyxes produced. Hindu Kush, Thai, and Mexican strains are some of the most psychoactive strains, and they are often characterized by large calyxes and seeds.

Calyx size appears to be an inherited trait in Cannabis. Completely acclimatized hybrid strains usually have many rather small calyxes, while imported strains with large calyxes retain that size when inbred.

Initial selection of large seeds increases the chance that offspring will be of the large-calyx variety. Aberrant calyx development occasionally results in double or fused calyxes, both of which may set seed. This phenomenon is most pronounced in strains from Thailand and India.

d) Color - The perception and interpretation of color in Cannabis floral clusters is heavily influenced by the imagination of the cultivator or breeder. A gold strain does not appear metallic any more than a red strain resembles a fire engine. Cannabis floral clusters are basically green, but changes may take place later in the season which alter the color to include various shades. The intense green of chlorophyll usually masks the color of accessory pigments, Chlorophyll tends to break down late in the season and

anthocyanin pigments also contained in the tissues are unmasked and allowed to show through. Purple, resulting from anthocyanin accumulation, is the most common color in living Cannabis, other than green. This color modification is usually triggered by seasonal change, much as the leaves of many deciduous trees change color in the fall. This does not mean, however, that expression of color is controlled by environment alone and is not an inheritable trait. For purple color to develop upon maturation, a strain must have the genetically controlled metabolic potential to pro duce anthocyanin pigments coupled with a responsiveness to environmental change such that anthocyanin pigments are unmasked and become visible. This also means that a strain could have the genes for expression of purple color but the color might never be expressed if the environmental conditions did not trigger anthocyanin pigmentation or chlorophyll breakdown. Colombian and Hindu Kush strains often develop purple coloration year after year when subjected to low night temperatures during maturation. Color changes will be discussed in more detail in Chapter IV-Maturation and Harvesting of Cannabis.

Carotenoid pigments are largely responsible for the yellow, orange, red, and brown colors of Cannabis. They also begin to show in the leaves and calyxes of certain strains as the masking green chlorophyll color fades upon maturation. Gold strains are those which tend to reveal underlying yellow and orange pigments as they mature. Red strains are usually closer to reddish brown in color, although certain carotenoid and anthocyanin pigments are nearly red and localized streaks of these colors occasionally appear in the petioles of very old floral clusters. Red color in pressed, imported tops is often a result of masses of reddish brown dried pistils.

Several different portions of floral cluster anatomy may change colors, and it is possible that different genes may control the coloring of these various parts.

The petioles, adaxial (top) surfaces, and abaxial (bot tom) surfaces of leaves, as well as the stems, calyxes, and pistils color differently in various strains.

Since most of the outer leaves are removed during manicuring, the color ex pressed by the calyxes and inner leaves during the late flowering stages will be all that remains in the final product. This is why strains are only considered to be truly purple or gold if the calyxes maintain those colors when dried. Anthocyanin accumulation in the stems is sometimes considered a sign of phosphorus deficiency but in most situations results from unharmful excesses of phosphorus or it is a genetic trait. Also, cold temperatures might interfere with phosphorus uptake resulting in a deficiency. Pistils in Hindu Kush strains are quite often magenta or pink in color when they first appear. They are viable at this time and turn reddish brown when they wither, as in most strains. Purple coloration usually indicates that pistillate plants are over-mature and cannabinoid biosynthesis is slowing down during cold autumn weather.

e) Cannabinoid Level - Breeding Cannabis for cannabinoid level has been accomplished by both licensed legitimate and clandestine researchers. Warmke (1942) and Warmke and Davidson (1943-44) showed that they could significantly raise or lower the cannabinoid level by selective breeding. Small (1975a) has divided genus Cannabis into four distinct chemotypes based on the relative amounts of THC and CBD. Recent research has shown that crosses between high THC: low CBD strains and low THC: high CBD strains yield offspring of cannabinoid content intermediate between the two parents. Beutler and der Marderosian (1978) analyzed the F1 offspring of the controlled cross C. Sativa (Mexico-high THC) X C. ruderalis (Russia-low THC) and found that they fell into two groups intermediate between the parents in THC level. This indicates that THC production is most likely controlled by more than one gene. Also the F1 hybrids of lower THC (resembling the staminate parent) were twice as frequent as the higher THC hybrids (resembling the pistillate parent). More re search is needed to learn if THC production in Cannabis is associated with the sexual type of the high THC parent or if high THC characteristics are recessive. According to Small (1979) the cannabinoid ratios of strains grown in northern climates are a reflection of the cannabinoid ratio of the pure,

imported, parental strain. This indicates that cannabinoid phenotype is genetically controlled, and the levels of the total cannabinoids are determined by environment. Complex highs produced by various strains of drug Cannabis may be blended by careful breeding to produce hybrids of varying psychoactivity, but the level of total psychoactivity is dependent on environment. This is also the telltale indication that unconscious breeding with undesirable low-THC parents could rapidly lead to the degeneration rather than improvement of a drug strain. It is obvious that individuals of fiber strains are of little if any use in breeding drug strains.

Breeding for cannabinoid content and the eventual characterization of varying highs produced by Cannabis is totally subjective guesswork without the aid of modern analysis techniques. A chromatographic analysis system would allow the selection of specific cannabinoid types, especially staminate pollen parents. Selection of staminate parents always presents a problem when breeding for cannabinoid content. Staminate plants usually express the same ratios of cannabinoids as their pistiliate counterparts but in much lower quantities, and they are rarely allowed to reach full maturity for fear of seeding the pistillate portion of the crop. A simple bioassay for THC content of staminate plants is performed by leaving a series of from three to five numbered bags of leaves and tops of various prospective pollen parents along with some rolling papers in several locations frequented by a steady repeating crowd of marijuana smokers. The bag completely consumed first can be considered the most desirable to smoke and possibly the most psychoactive. It would be impossible for one per son to objectively select the most psychoactive staminate plant since variation in the cannabinoid profile is subtle. The bioassay reported here is in effect an unstructured panel evaluation which averages the opinions of unbiased testers who are exposed to only a few choices at a time. Such bioassay results can enter into selecting the staminate parent.

It is difficult to say how many genes might control THC-acid synthesis. Genetic control of the

biosynthetic pathway could occur at many points through the action of enzymes controlling each individual reaction. It is generally accepted that drug strains have an enzyme system which quickly converts CBD-acid to THC-acid, favoring THC-acid accumulation. Fiber strains lack this enzyme activity, so CBD-acid accumulalion is favored since there is little con version to THC-acid. These same enzyme systems are probably also sensitive to changes in heat and light.

It is supposed that variations in the type of high associated with different strains of Cannabis result from varying levels of cannabinoids. THC is the primary psycho active ingredient which is acted upon synergistically by small amounts of CBN, CBD, and other accessory cannabinoids. Terpenes and other aromatic constituents of Cannabis might also potentiate or suppress the effect of THC. We know that cannabinoid levels may be used to establish cannabinoid phenotypes and that these phenotypes are passed on from parent to offspring. Therefore, cannabinoid levels are in part determined by genes. To accurately characterize highs from various individuals and establish criteria for breeding strains with particular cannabinoid contents, an accurate and easy method is needed for measuring cannabinoid levels in prospective parents. Inheritance and expression of cannabinoid chemotype is certainly complex.

f) Taste and Aroma - Taste and aroma are closely linked.

As our senses for differentiating taste and aroma are connected, so are the sources of taste and aroma in Cannabis. Aroma is produced primarily by aromatic terpenes produced as components of the resin secreted by glandular trichomes on the surface of the calyxes and subtending leaflets. When a floral cluster is squeezed, the resinous heads of glandular trichomes rupture and the aromatic terpenes are exposed to the air. There is often a large difference between the aroma of fresh and dry floral clusters. This is explained by the polymerization (joining together in a chain) of many of the smaller molecules

of aromatic terpenes to form different aromatic and nonaromatic terpene polymers. This happens as Cannabis resins age and mature, both while the plant is growing and while curing after harvest. Additional aromas may interfere with the primary terpenoid components, such as ammonia gas and other gaseous products given off by the curing, fermentation or spoilage of the tissue (non-resin) portion of the floral clusters.

A combination of at least twenty aromatic terpenes (103 are known to occur in Cannabis) and other aromatic compounds control the aroma of each plant. The production of each aromatic compound may be influenced by many genes; therefore, it is a complex matter to breed Cannabis for aroma. Breeders of perfume roses often are amazed at the complexity of the genetic control of aroma, Each strain, however, has several characteristic aromas, and these are occasionally transmitted to hybrid offspring such that they resemble one or both parents in aroma. Many times breeders complain that their strain has lost the de sired aromatic characteristics of the parental strains. Fixed hybrid strains will develop a characteristic aroma that is hereditary and often true-breeding. The cultivator with preservation of a particular aroma as a goal can clone the individual with a desired aroma in addition to breeding it. This is good insurance in case the aroma is lost in the off spring by segregation and recombination of genes.

The aromas of fresh or dried clusters are sampled and compared in such a way that they are separated to avoid confusion. Each sample is placed in the corner of a twice-folded, labeled piece of unscented writing paper at room temperature (above 650). A light squeeze will release the aromatic principles contained within the resin exuded by the ruptured glandular trichome head. When sampling, never squeeze a floral cluster directly, as the resins will ad here to the fingers and bias further sampling. The folded paper conveniently holds the floral cluster, avoids confusion during sampling, and contains the aromas as a glass does in wine tasting.

Taste is easily sampled by loosely rolling dried floral clusters in a cigarette paper and inhaling to draw a taste across the tongue. Samples should be approximately the same size.

Taste in Cannabis is divided into three categories according to usage: the taste of the aromatic components carried by air that passes over the Cannabis when it is in haled without being lighted; the taste of the smoke from burning Cannabis; and the taste of Cannabis when it is consumed orally. These three are separate entities.

The terpenes contained in a taste of unlighted Cannabis are the same as those sensed in the aroma, but perceived through the sense of taste instead of smell. Orally ingested Cannabis generally tastes bitter due to the vegetative plant tissues, but the resin is characteristically spicy and hot, somewhat like cinnamon or pepper. The taste of Cannabis smoke is determined by the burning tissues and vaporizing terpenes. These terpenes may not be detected in the aroma and unlighted taste.

Biosynthetic relationships between terpenes and cannabinoids have been firmly established. Indeed, cannabinoids are synthesized within the plant from terpene precursors. It is suspected that changes in aromatic terpene levels parallel changes in cannabinoid levels during maturation. As connections between aroma and psycho activity are uncovered, the breeder will be better able to make field selections of prospective high-THC parents without complicated analysis.

g) Persistence of Aromatic Principles and Cannabinoids - Cannabis resins deteriorate as they age, and the aromatic principles and cannabinoids break down slowly until they are hardly noticeable. Since fresh Cannabis is only available once a year in temperate regions, an important breeding goal has been a strain that keeps well when packaged. Packageability and shelf life are important considerations in the breeding of fresh fruit species and will prove equally important if trade in Cannabis develops after legalization.

h) Trichome Type - Several types of trichomes are present on the epidermal surfaces of Cannabis. Several of these trichomes are glandular and secretory in nature and are divided into bulbous, capitate sessile, and capitate stalked types. Of these, the capitate stalked glandular trichomes are apparently responsible for the intense secretion of cannabinoid laden resins. Plants with a high density of capitate stalked trichomes are a logical goal for breeders of drug Cannabis. The number and type of trichomes is easily characterized by observation with a small hand lens (lOX to 50X). Recent research by V. P. Soroka (1979) concludes that a positive correlation exists between the number of glandular trichomes on leaves and calyxes and the various cannabinoid contents of the floral clusters. In other words, many capitate stalked trichomes means higher THC levels.

i) Resin Quantity and Quality - Resin production by the glandular trichomes varies. A strain may have many glandular trichomes but they may not secrete very much resin. Resin color also varies from strain to strain. Resin heads may darken and become more opaque as they mature, as suggested by several authors. Some strains, however, pro duce fresh resins that are transparent amber instead of clear and colorless, and these are often some of the most psycho active strains. Transparent resins, regardless of color, are a sign that the plant is actively carrying out resin biosynthesis. When biosynthesis ceases, resins turn opaque as cannabinoid and aromatic levels decline. Resin color is certainly an indication of the conditions inside the resin head, and this may prove to be another important criterion for breeding.

j) Resin Tenacity - For years strains have been bred for hashish production. Hashish is formed from detached resin heads. In modern times it might be feasible to breed a strain with high resin production that gives up its precious covering of resin heads with only moderate shaking, rather than the customary flailing that also breaks up the plant. This would facilitate hashish production. Strains that are bred for use as marijuana would benefit from extremely

tenacious resin heads that would not fall off during packaging and shipment.

k) Drying and Curing Rate - The rate and extent to which Cannabis dries is generally determined by the way it is dried, but, all conditions being the same, some strains dry much more rapidly and completely than others. It is assumed that resin has a role in preventing desiccation and high resin content might retard drying. However, it is a misconception that resin is secreted to coat and seal the surface of the calyxes and leaves. Resin is secreted by glandular trichomes, but they are trapped under a cuticle layer surrounding the head cells of the trichome holding the resin away from the surface of the leaves. There it would rarely if ever have a chance to seal the surface of the epidermal layer and prevent the transpiration of water. It seems that an alternate reason must be found for the great variations in rate and extent of drying. Strains may be bred that dry and cure rapidly to save valuable time.

1) Ease of Manicuring - One of the most time-consuming aspects of commercial drug Cannabis production is the seemingly endless chore of manicuring, or removing the larger leaves from the floral clusters. These larger outer leaves are not nearly as psychoactive as the inner leaves and calyxes, so they are usually removed before selling as marijuana. Strains with fewer leaves obviously require less time to manicure. Long petioles on the leaves facilitate removal by hand with a small pair of scissors. If there is a marked size difference between very large outer leaves and tiny, resinous inner leaves it is easier to manicure quickly because it is easier to see which leaves to remove.

m) Seed Characteristics - Seeds may be bred for many characteristics including size, oil content, and protein con tent. Cannabis seed is a valuable source of drying oils, and Cannabis-seed cake is a fine feed for ranch animals. Higher-protein varieties may be developed for food. Also, seeds are selected for rapid germination rate.

n) Maturation - Cannabis strains differ greatly as to when they mature and how they respond to changing environment. Some strains, such as Mexican and Hindu Kush, are famous for early maturation, and others, such as Colombian and Thai, are stubborn in maturing and nearly always finish late, if at all. Imported strains are usually characterized as either early, average, or late in maturing; however, a particular strain may produce some individuals which mature early and others which mature late. Through selection, breeders have, on the one hand, developed strains that mature in four weeks, outdoors under temperate conditions; and on the other hand, they have developed green house strains that mature in up to four months in their protected environment. Early maturation is extremely advantageous to growers who live in areas of late spring and early fall freezes. Consequently, especially early-maturing plants are selected as parents for future early-maturing strains.

o) Flowering - Once a plant matures and begins to bear flowers it may reach peak floral production in a few weeks, or the floral clusters may continue to grow and develop for several months. The rate at which a strain flowers is independent of the rate at which it matures, so a plant may wait until late in the season to flower and then grow extensive, mature floral clusters in only a few weeks.

p) Ripening - Ripening of Cannabis flowers is the final step in their maturation process Floral clusters will usually mature and ripen in rapid succession, but sometimes large floral clusters will form and only after a period of apparent hesitation will the flowers begin to produce resin and ripen. Once ripening starts it usually spreads over the entire plant, but some strains, such as those from Thailand, are known to ripen a few floral clusters at a time over several months. Some fruit trees are similarly everbearing with a yearlong season of production. Possibly Cannabis strains could be bred that are true everbearing perennials that continue to flower and mature consistently all year long.

q) Cannabinoid Profile - It is supposed that variations in the type of high associated with different strains of

Cannabis result from varying levels of cannabinoids. THC is the primary psychoactive ingredient which is acted upon synergistically by small amounts of CBN, CBD, and other accessory cannabinoids. We know that cannabinoid levels may be used to establish cannabinoid phenotypes and that these phenotypes are passed on from parent to offspring. Therefore, cannabinoid levels are in part determined by genes. To accurately characterize highs from various individuals and establish criteria for breeding strains with particular cannabinoid contents, an accurate and easy method is necessary for measuring cannabinoid levels in prospective parents.

Various combinations of these traits are possible and inevitable. The traits that we most often see are most likely dominant and any effort to alter genetics and improve Cannabis strains are most easily accomplished by concentrating on the major phenotypes for the most important traits. The best breeders set high goals of a limited scope and adhere to their ideals.

6. Gross Phenotypes of Cannabis Strains

The gross phenotype or general growth form is deter mined by size, root production, branching pattern, sex, maturation, and floral characteristics. Most imported varieties have characteristic gross phenotypes although there tend to be occasional rare examples of almost every phenotype in nearly every variety. This indicates the complexity of genetic control determining gross phenotype. Hybrid crosses between imported pure varieties were the beginning of nearly every domestic strain of Cannabis. In hybrid crosses, some dominant characteristics from each parental variety are exhibited in various combinations by the F1 offspring. Nearly all of the offspring will resemble both parents and very few will resemble only one parent. This sounds like it is saying a lot, but this F1 hybrid generation is far from true-breeding and the subsequent F2 generation will exhibit great variation, tending to look more like one or the other of the original imported parental varieties, and will also exhibit recessive traits not apparent in either of the original parents. If the F1 offspring are desirable plants it will be difficult to continue the hybrid traits in subsequent generations. Enough of the original F1 hybrid seeds are produced so they may be

used year after year to pro-duce uniform crops of desirable plants.

Phenotypes and Characteristics

of Imported Strains

Following is a list of gross phenotypes and characteristics for many imported strains of Cannabis.

1. Fiber Strain Gross Phenotypes (hemp types)

2. Drug Strain Gross Phenotypes

a) Colombia - highland, lowland (marijuana)

b) Congo - (marijuana)

c) Hindu Kush - Afghanistan and Pakistan (hashish)

d) Southern India - (ganja marijuana)

e) Jamaica - Carribean hybrids

f) Kenya - Kisumu (dagga marijuana)

g) Lebanon - (hashish)

h) Malawi, Africa - Lake Nyasa (dagga marijuana)

i) Mexico - Michoacan, Oaxaca, Guerrero (marijuana)

j) Morocco - Rif mountains (kif marijuana and hashish)

h) Nepal - wild (ganja marijuana and hashish)

1) Russian - ruderalis (uncultivated)

m) South Africa - (dagga marijuana)

n) Southeast Asia - Cambodia, Laos, Thailand, Vietnam (ganja marijuana)

3. Hybrid Drug Phenotypes

a) Creeper Phenotype

b) Huge Upright Phenotype

In general the F1 and F2 pure-bred offspring of these imported varieties are more similar to each other than they are to other varieties and they are termed pure strains.

However, it should be remembered that these are average. Gross phenotypes and recessive variations within each trait will occur. In addition, these representations are based on unpruned plants growing in ideal conditions and stress will alter the gross phenotype. Also, the protective environment of a greenhouse tends to obscure the difference between different strains. This section presents information that is used in the selection of pure strains for breeding.

1. Fiber Strain Gross Phenotypes Fiber strains are characterized as tall, rapidly maturing, limbless plants which are often monoecious. This growth habit has been selected by generations of fiber-producing farmers to facilitate forming long fibers through even growth and maturation. Monoecious strains mature more evenly than dioecious strains, and fiber crops are usually not grown long enough to set seed which interferes with fiber production. Most varieties of fiber Cannabis originate in the northern temperate climates of Europe, Japan, China and North America. Several strains have been selected from the prime hemp growing areas and offered commercially over the last fifty years in both Europe and America. Escaped fiber strains of the midwestern United States are usually tall, skinny, relatively poorly branched, weakly flowered, and low in cannabinoid production. They represent an escaped race of Cannabis sativa hemp. Most fiber strains contain CBD as the primary cannabinoid and little if any THC.

2. Drug Strain Gross Phenotypes Drug strains are characterized by Delta1-THC as the primary cannabinoid, with low levels of other accessory cannabinoids such as THCV, CBD, CBC, and CBN. This results from selective breeding for high potency or natural selection in niches where Delta1-THC biosynthesis favors survival.

a) Colombia - (0 to 10 north latitude)

Colombian Cannabis originally could be divided into two basic strains: one from the low-altitude humid coastal areas along the Atlantic near Panama, and the other from the more arid mountain areas inland from Santa Marta. More recently, new areas of cultivation in the interior plateau of southern central Colombia and the highland valleys stretching southward from the Atlantic coast

have become the primary areas of commercial export Cannabis cultivation. Until recent years high quality Cannabis was available through the black market from both coastal and highland Colombia. Cannabis was introduced to Colombia just over 100 years ago, and its cultivation is deeply rooted in tradition. Cultivation techniques often involve transplanting of selected seedlings and other individual attention. The production of "la mona amarilla" or gold buds is achieved by girdling or removing a strip of bark from the main stem of a nearly mature plant, thereby restricting the flow of water, nutrients, and plant products. Over several days the leaves dry up and fall off as the flowers slowly die and turn yellow. This produces the highly prized "Colombian gold" so prevalent in the early to middle 1970s (Partridge 1973). Trade names such as "punta roja" (red tips [pistils]), "Cali Hills," "choco," "lowland," "Santa Marta gold," and "purple" give us some idea of the color of older varieties and the location of cultivation.

In response to an incredible demand by America for Cannabis, and the fairly effective control of Mexican Cannabis importation and cultivation through tightening border security and the use of Paraquat, Colombian farmers have geared up their operations. Most of the marijuana smoked in America is imported from Colombia. This also means that the largest number of seeds available for domes tic cultivation also originate in Colombia. Cannabis agri-business has squeezed out all but a few small areas where labor-intensive cultivation of high quality drug Cannabis such as "Ia mona amarilla" can continue. The fine marijuana of Colombia was often seedless, but commercial grades are nearly always well seeded. As a rule today, the more remote highland areas are the centers of commercial agriculture and few of the small farmers remain. It is thought that some highland farmers must still grow fine Cannabis, and occasional connoisseur crops surface. The older seeds from the legendary Colombian strains are now highly prized by breeders. In the heyday of "Colombian gold" this fine cerebral marijuana was grown high in the mountains. Humid lowland marijuana was characterized by stringy, brown, fibrous floral clusters of sedative narcotic high. Now highland marijuana has become the commercial product and is characterized by leafy brown floral clusters and sedative effect. Many of the unfavorable characteristics of imported Colombian Cannabis result from hurried commercial agricultural techniques combined with poor curing and storage. Colombian seeds still contain genes favoring vigorous growth and high THC production. Colombian strains also contain high levels of CBD and CBN, which could account for sedative highs and result from poor curing and storage techniques. Domestic Colombian strains usually lack CBD and CBN. The

commercial Cannabis market has brought about the eradication of some local strains by hybridizing with commercial strains.

Colombian strains appear as relatively highly branched conical plants with a long upright central stem, horizontal limbs and relatively short internodes. The leaves are characterized by highly serrated slender leaflets (7-11) in a nearly complete to overlapping circular array of varying shades of medium green. Colombian strains usually flower late in temperate regions of the northern hemisphere and may fail to mature flowers in colder climates. These strains favor the long equatorial growing seasons and often seem insensitive to the rapidly decreasing daylength during autumn in temperate latitudes. Because of the horizontal branching pattern of Colombian strains and their long growth cycle, pistillate plants tend to produce many flowering clusters along the entire length of the stem back to the central stalk. The small flowers tend to produce small, round, dark, mottled, and brown seeds. Imported and domestic Colombian Cannabis often tend to be more sedative in psychoactivity than other strains. This may be caused by the synergistic effect of THC with higher levels of CBD or CBN. Poor curing techniques on the part of Colombian farmers, such as sun drying in huge piles resembling com post heaps, may form CBN as a degradation product of THC. Colombian strains tend to make excellent hybrids with more rapidly maturing strains such as those from Central and North America.

b) Congo - (5 north to 5 south latitude)

Most seeds are collected from shipments of commercial grade seeded floral clusters appearing in Europe.

c) Hindu Kush Range - Cannabis indica (Afghanistan and Pakistan) - (30 to 37 north latitude)

This strain from the foothills (up to 3,200 meters [10,000 feetj) of the Hindu Kush range is grown in small rural gardens, as it has been for hundreds of years, and is used primarily for the production of hashish. In these areas hashish is usually made from the resins covering the pistil late calyxes and associated leaflets. These resins are re moved by shaking and crushing the flowering tops over a silk screen and collecting the dusty resins that fall off the plants. Adulteration and pressing usually follow in the production of commercial hashish. Strains from this area are often used as type examples for Cannabis indica. Early maturation and the belief by clandestine cultivators that this strain may be exempt from laws controlling Cannabis sativa and indeed may be legal, has resulted

in its proliferation throughout domestic populations of "drug" Cannabis. Names such as "hash plant" and "skunk weed" typify its acrid aroma reminiscent of "primo" hashish from the high valleys near Mazar-i-Sharif, Chitral, and Kandahar in Afghanistan and Pakistan.

This strain is characterized by short, broad plants with thick, brittle woody stems and short internodes. The main stalk is usually only four to six feet tall, but the relatively unbranched primary limbs usually grow in an upright fashion until they are nearly as tall as the central stalk and form a sort of upside-down conical shape. These strains are of medium size, with dark green leaves having 5 to 9 very wide, coarsely serrated leaflets in a circular array. The lower leaf surface is often lighter in color than the upper surface. These leaves have so few broad coarse leaflets that they are often compared to a maple leaf. Floral clusters are dense and appear along the entire length of the primary limbs as very resinous leafy balls. Most plants produce flowering clusters with a low calyx-to-leaf ratio, but the inner leaves associated with the calyxes are usually liberally encrusted with resin. Early maturation and extreme resin production is characteristic of these strains. This may be the result of acclimatization to northern temperate latitudes and selection for hashish production. The acrid smell associated with strains from the Hindu Kush appears very early in the seedling stage of both staminate and pistillate individuals and continues throughout the life of the plant. Sweet aromas do often develop but this strain usually loses the sweet fragrance early, along with the clear, cerebral psychoactivity.

Short stature, early maturation, and high resin production make Hindu Kush strains very desirable for hybridizing and indeed they have met with great popularity. The gene pool of imported Hindu Kush strains seems to be dominant for these desirable characteristics and they seem readily passed on to the F1 hybrid generation. A fine hybrid may result from crossing a Hindu Kush variety with a late-maturing, tall, sweet strain from Thailand, India, or Nepal. This produces hybrid offspring of short stature, high resin content, early maturation, and sweet taste that will mature high quality flowers in northern climates. Many hybrid crosses of this type are made each year and are currently cultivated in many areas of North America. Hindu Kush seeds are usually large, round, and dark grey or black in coloring with some mottling.

d) India Centra1 Southern - Kerala, Mysore, and Madras regions (10 to 20 north latitude)

Ganja (or flowering Cannabis tops) has been grown in India for hundreds of years. These strains are usually grown in a seedless fashion and are cured, dried, and smoked as marijuana instead of being converted to hashish as in many Central Asian areas. This makes them of considerable interest to domestic Cannabis cultivators wishing to reap the benefits of years of selective breeding for fine ganja by Indian farmers. Many Europeans and Americans now live in these areas of India and ganja strains are finding their way into domestic American Cannabis crops.

Ganja strains are often tall and broad with a central stalk up to 12 feet tall and spreading highly-branched limbs. The leaves are medium green and made up of 7 to 11 leaf lets of moderate size and serration arranged in a circular array. The frond-like limbs of ganja strains result from extensive compound branching so that by the time floral clusters form they grow from tertiary or quaternary limbs. This promotes a high yield of floral clusters which in ganja strains tend to be small, slender, and curved. Seeds are usually small and dark. Many spicy aromas and tastes occur in Indian ganja strains and they are extremely resinous and psychoactive. Medicinal Cannabis of the late 1800s and early 1900s was usually Indian ganja.

e) Jamaica - (18 north latitude)

Jamaican strains were not uncommon in the late 1960s and early 1970s but they are much rarer today. Both green and brown varieties are grown in Jamaica. The top-of-the-line seedless smoke is known as the "lamb's bread" and is rarely seen outside Jamaica. Most purported Jamaican strains appear stringy and brown much like low land or commercial Colombian strains. Jamaica's close proximity to Colombia and its position along the routes of marijuana smuggling from Colombia to Florida make it likely that Colombian varieties now predominate in Jamaica even if these varieties were not responsible for the original Jamaican strains. Jamaican strains resemble Colombian strains in leaf shape, seed type and general morphology but they tend to be a little taller, thinner, and lighter green. Jamaican strains produce a psychoactive effect of a particularly clear and cerebral nature, unlike many Colombian strains. Some strains may also have come to Jamaica from the Caribbean coast of Mexico, and this may account for the introduction of cerebral green strains.

f) Kenya - Kisumu (5 north to 5 south latitude)

Strains from this area have thin leaves and vary in color from light to dark green. They are characterized by cerebral psychoactivity and sweet taste. Hermaphrodites are common.

g) Lebanon - (34 north latitude)

Lebanese strains are rare in domestic Cannabis crops but do appear from time to time. They are relatively short and slender with thick stems, poorly developed limbs, and wide, medium-green leaves with 5 to 11 slightly broad leaflets. They are often early-maturing and seem to be quite leafy, reflecting a low calyx-to-leaf ratio. The calyxes are relatively large and the seeds flattened, ovoid and dark brown in color. As with Hindu Kush strains, these plants are grown for the production of screened and pressed hashish, and the calyx-to-leaf ratio may be less important than the total resin production for hashish making. Lebanese strains resemble Hindu Kush varieties in many ways and it is likely that they are related.

h) Malawi, Africa - (10 to 15 south latitude)

Malawi is a small country in eastern central Africa bordering Lake Nyasa. Over the past few years Cannabis from Malawi has appeared wrapped in bark and rolled tightly, approximately four ounces at a time. The nearly seedless flowers are spicy in taste and powerfully psycho active. Enthusiastic American and European Cannabis cultivators immediately planted the new strain and it has be come incorporated into several domestic hybrid strains. They appear as a dark green, large plant of medium height and strong limb growth. The leaves are dark green with coarsely serrated, large, slender leaflets arranged in a narrow, drooping, hand-like array. The leaves usually lack serrations on the distal (tip portion) 20% of each leaflet. The mature floral clusters are sometimes airy, resulting from long internodes, and are made up of large calyxes and relatively few leaves. The large calyxes are very sweet and resinous, as well as extremely psychoactive. Seeds are large, shortened, flattened, and ovoid in shape with a dark grey or reddish brown, mottled perianth or seed coat. The caruncle or point of attachment at the base of the seed is uncommonly deep and usually is surrounded by a sharp edged lip. Some individuals turn a very light yellow green in the flowering clusters as they mature under exposed conditions. Although they mature relatively late, they do seem to have met with acceptance in Great Britain and North America as drug strains. Seeds of many strains appear in small batches of low-quality African marijuana easily available in Amsterdam and other European cities. Phenotypes vary

considerably, however, many are similar in appearance to strains from Thailand.

i) Mexico - (15 to 27 north latitude)

Mexico had long been the major source of marijuana smoked in America until recent years. Efforts by the border patrols to stop the flow of Mexican marijuana into the United States were only minimally effective and many varieties of high quality Mexican drug Cannabis were continually available. Many of the hybrid strains grown domestic ally today originated in the mountains of Mexico. In recent years, however, the Mexican government (with monetary backing by the United States) began an intensive pro gram to eradicate Cannabis through the aerial spraying of herbicides such as Paraquat. Their program was effective, and high quality Mexican Cannabis is now rarely available. It is ironic that the NIMH (National Institute of Mental Health) is using domestic Mexican Cannabis strains grown in Mississippi as the pharmaceutical research product for chemotherapy and glaucoma patients. In the prime of Mexican marijuana cultivation from the early 1960s to the middle 1970s, strains or "brands" of Cannabis were usually affixed with the name of the state or area where they were grown. Hence names like "Chiapan," "Guerreran," "Nayarit," "Michoacan," "Oaxacan," and "Sinaloan" have geo graphic origins behind their common names and mean something to this very day. All of these areas are Pacific coastal states extending in order from Sinaloa in the north at 27; through Nayarit, Jalisco, Michoacan, Guerrero, and Oaxaca; to Chiapas in the south at 15 - All of these states stretch from the coast into the mountains where Cannabis is grown.

Strains from Michoacan, Guerrero, and Oaxaca were the most common and a few comments may be ventured about each and about Mexican strains in general.

Mexican strains are thought of as tall, upright plants of moderate to large size with light to dark green, large leaves. The leaves are made up of long, medium width, moderately serrated leaflets arranged in a circular array. The plants mature relatively early in comparison to strains from Colombia or Thailand and produce many long floral clusters with a high calyx-to-leaf ratio and highly cerebral psychoactivity. Michoacan strains tend to have very slender leaves and a very high calyx-to-leaf ratio as do Guerreran strains, but Oaxacan strains tend to be broader-leafed, often with leafier floral clusters. Oaxacan strains are generally the largest and grow vigorously, while Michoacan strains are smaller and more delicate. Guerreran strains are often short and develop long,

upright lower limbs. Seeds from most Mexican strains are fairly large, ovoid, and slightly flattened with a light colored grey or brown, unmottled perianth. Smaller, darker, more mottled seeds have appeared in Mexican marijuana during recent years. This may indicate that hybridization is taking place in Mexico, possibly with introduced seed from the largest seed source in the world, Colombia. No commercial seeded Cannabis crops are free from hybridization and great variation may occur in the offspring. More recently, large amounts of hybrid domestic seed have been introduced into Mexico. It is not uncommon to find Thai and Afghani phenotypes in recent shipments of Cannabis from Mexico.

j) Morocco, Rif Mountains - (35 north latitude)

The Rif mountains are located in northernmost Morocco near the Mediterranean Sea and range up to 2,500 meters (8,000 feet). On a high plateau surrounding the city of Ketama grows most of the Cannabis used for kif floral clusters and hashish production. Seeds are broad-sown or scattered on rocky terraced fields in the spring, as soon as the last light snows melt, and the mature plants are harvested in late August and September. Mature plants are usually 1 to 2 meters (4 to 6 feet) tall and only slightly branched. This results from crowded cultivation techniques and lack of irrigation. Each pistillate plant bears only one main terminal flower cluster full of seeds. Few staminate plants, if any, are pulled to prevent pollination. Although Cannabis in Morocco was originally cultivated for floral clusters to be mixed with tobacco and smoked as kif, hashish production has begun in the past 30 years due to Western influence. In Morocco, hashish is manufactured by shaking the entire plant over a silk screen and collecting the powdery resins that pass through the screen. It is a matter of speculation whether the original Moroccan kif strains might be extinct. It is reported that some of these strains were grown for seedless flower production and areas of Morocco may still exist where this is the tradition.

Because of selection for hashish production, Moroccan strains resemble both Lebanese and Hindu Kush strains in their relatively broad leaves, short growth habit, and high resin production. Moroccan strains are possibly related to these other Cannabis indica types.

k) Nepal - (26 to 30 north latitude)

Most Cannabis in Nepal occurs in wild stands high in the Himalayan foothills (up to 3,200 meters [10,000 feet]). Little Cannabis is cultivated, and it is from select wild plants that most

Nepalese hashish and marijuana originate. Nepalese plants are usually tall and thin with long, slightly branched limbs. The long, thin flowering tops are very aromatic and reminiscent of the finest fresh "temple ball" and "finger" hashish hand-rubbed from wild plants. Resin production is abundant and psychoactivity is high Few Nepalese strains have appeared in domestic Cannabis crops but they do seem to make strong hybrids with strains from domestic sources and Thailand.

l) Russian - (35 to 60 north latitude) Cannabis ruderalis (uncultivated)

Short stature (10 to 50 centimeters [3 to 18 inches]) and brief life cycle (8 to 10 weeks), wide, reduced leaves and specialized seeds characterize weed Cannabis of Russia. Janischewsky (1924) discovered weedy Cannabis and named it Cannabis ruderalis. Ruderalis could prove valuable in breeding rapidly maturing strains for commercial use in temperate latitudes. It flowers when approximately 7 weeks old without apparent dependence on daylength. Russian Cannabis ruderalis is nearly always high in CBD and low in THC.

m) South Africa - (22 to 35 south latitude)

Dagga of South Africa is highly acclaimed. Most seeds have been collected from marijuana shipments in Europe. Some are very early-maturing (September in northern latitudes) and sweet smelling. The stretched light green floral clusters and sweet aroma are comparable to Thai strains.

n) Southeast Asia - Cambodia, Laos, Thailand and Vietnam (10 to 20 north latitude)

Since American troops first returned from the war in Vietnam, the Cambodian, Laotian, Thai, and Vietnamese strains have been regarded as some of the very finest in the world. Currently most Southeast Asian Cannabis is produced in northern and eastern Thailand. Until recent times, Cannabis farming has been a cottage industry of the northern mountain areas and each family grew a small garden. The pride of a farmer in his crop was reflected in the high quality and seedless nature of each carefully wrapped Thai stick. Due largely to the craving of Americans for exotic marijuana, Cannabis cultivation has become a big business in Thailand and many farmers are growing large fields of lower quality Cannabis in the eastern lowlands. It is suspected that other Cannabis strains, brought to Thailand to replenish local strains and begin large

plantations, may have hybridized with original Thai strains and altered the resultant genetics. Also, wild stands of Cannabis may now be cut and dried for export.

Strains from Thailand are characterized by tall meandering growth of the main stalk and limbs and fairly extensive branching. The leaves are often very large with 9 to 11 long, slender, coarsely serrated leaflets arranged in a drooping hand like array. The Thai refer to them as "alligator tails" and the name is certainly appropriate.

Most Thai strains are very late-maturing and subject to hermaphrodism. It is not understood whether strains from Thailand turn hermaphrodite as a reaction to the extremes of northern temperate weather or if they have a genetically controlled tendency towards hermaphrodism. To the dismay of many cultivators and researchers, Thai strains mature late, flower slowly, and ripen unevenly. Retarded floral development and apparent disregard for changes in photoperiod and weather may have given rise to the story that Cannabis plants in Thailand live and bear flowers for years. Despite these shortcomings, Thai strains are very psychoactive and many hybrid crosses have been made with rapidly maturing strains, such as Mexican and Hindu Kush, in a successful attempt to create early-maturing hybrids of high psychoactivity and characteristic Thai sweet, citrus taste. The calyxes of Thai strains are very large, as are the seeds and other anatomical features, leading to the misconception that strains may be polyploid. No natural polyploidy has been discovered in any strains of Cannabis though no one has ever taken the time to look thoroughly. The seeds are very large, ovoid, slightly flattened, and light brown or tan in color. The perianth is never mottled or striped except at the base. Greenhouses prove to be the best way to mature stubborn Thai strains in temperate climes.

3. Hybrid Drug Phenotypes

a) Creeper Phenotype - This phenotype has appeared in several domestic Cannabis crops and it is a frequent phenotype in certain hybrid strains. It has not yet been deter mined whether this trait is genetically controlled (dominant or recessive), but efforts to develop a true-breeding strain of creepers are meeting with partial success. This phenotype appears when the main stalk of the seedling has grown to about 1 meter (3 feet) in height. It then begins to bend at approximately the middle of the stalk, up to 700 from the vertical, usually in the direction of the sun. Sub sequently, the first limbs sag until they touch the ground and begin to grow back up. In extremely

loose mulch and humid conditions the limbs will occasionally root along the bottom surface. Possibly as a result of increased light exposure, the primary limbs continue to branch once or twice, creating wide frond-like limbs of buds resembling South Indian strains. This phenotype usually produces very high flower yields. The leaves of these creeper phenotype plants are nearly always of medium size with 7-11 long, narrow, highly serrated leaflets.

b) Huge Upright Phenotype - This phenotype is characterized by medium size leaves with narrow, highly serrated leaflets much like the creeper strains, and may also be an acclimatized North American phenotype. In this phenotype, however, a long, straight central stalk from 2 to 4 meters (6.5 to 13 feet) tall forms and the long, slender primary limbs grow in an upright fashion until they are nearly as tall or occasionally taller than the central stalk. This strain resembles the Hindu Kush strains in general shape, except that the entire domestic plant is much larger than the Hindu Kush with long, slender, more highly branched primary limbs, much narrower leaflets, and a higher calyx-to-leaf ratio. These huge upright strains are also hybrids of many different imported strains and no specific origin may be determined.

The preceding has been a listing of gross phenotypes for several of the many strains of Cannabis occurring world wide. Although many of them are rare, the seeds appear occasionally due to the extreme mobility of American and European Cannabis enthusiasts. As a consequence of this extreme mobility, it is feared that many of the world's finest strains of Cannabis have been or may be lost forever due to hybridization with foreign Cannabis populations and the socio-economic displacement of Cannabis cultures worldwide. Collectors and breeders are needed to preserve these rare and endangered gene pools before it is too late.

Various combinations of these traits are possible and inevitable. The traits that we most often see are most likely dominant and the improvement of Cannabis strains through breeding is most easily accomplished by concentrating on the dominant phenotypes for the most important traits. The best breeders set high goals of limited scope and ad here to their ideals.

Chapter 4 - Maturation and Harvesting of Cannabis

To everything there is a season, and a time to every purpose under heaven:
A time to be born, and a time to die;
a time to plant, and a time to pluck up
that which is planted,

- Ecciesiastes 3:1-2

Maturation

The maturation of Cannabis is normally annual and its timing is influenced by the age of the plant, changes in photoperiod, and other environmental conditions. When a plant reaches an adequate age for flowering (about two months) and the nights lengthen following the summer solstice (June 21-22), flowering begins. This is the triggering of the reproductive phase of the life cycle which is followed by senescence and eventual death. The leaves of Cannabis plants form fewer leaflets during flowering until the floral clusters are formed of trileaflet and mono-leaflet leaves. This is a reversal of the heteroblastic (variously shaped) trend of increased leaflet number through the pre-floral stage.

The staminate and pistillate sexes of the same strain mature at different rates. Staminate plants are usually the first to begin flowering and releasing pollen. In fact, much pollen is released when the pistillate plants show only a few pairs of primordial flowers. It would seem more effective for the staminate plant to release pollen when the pistillate plants are in heavy flower to ensure good seed production. Upon deeper investigation, however, it becomes obvious that early pollination is advantageous to survival. Pollinations that take place early form seeds that ripen in the warm days of summer when the pistillate plant is healthy and there is less chance of frost damage or predation by herbivores. If conditions are favorable, the staminate plant will continue to produce pollen for some time and will also fertilize many new pistillate flowers as they appear. After a month or more of shedding pollen the staminate plants enter senescence. This period is

marked by the yellowing and dropping of the foliage leaves, followed by diminished flower and pollen production. Eventually, all the leaves drop, and the spent, lifeless stamens hang in the breeze until fungi and bacteria return them to the soil.

Pistillate plants continue to develop up to three months longer as they mature seeds. As the calyxes of the first flowers to be pollinated dry out, each releases a single seed which falls to the ground. Since new pistillate flowers are continually produced and fertilized, there are nearly always seeds ranging in maturity from freshly fertilized ovules to large, dark, mature seeds. In this way the plant is able to take advantage of favorable conditions throughout several months. The effectiveness of this type of reproduction is demonstrated by the spread of escaped Cannabis strains in the midwestern United States. In these areas Can nabis abounds and multiplies each year, through the timely dehiscence of millions of pollen grains and the fertilization of thousands of pistillate flowers, resulting in thousands of viable seeds from each pistillate plant. As the pistillate plant senesces, the leaves turn yellow and drop, along with the remaining mature seeds. The rest of the plant eventually dies and decomposes.

Although the staminate plants begin to release pollen before the pistillate plant has begun to form floral clusters, pistillate plants actually differentiate sexually and form a few viable flowers long before most of the staminate plants begin to release pollen. This ensures that the first pollen released has a chance to fertilize at least a few flowers and produce seeds. The production of prominent pistils makes pistillate plants the first to be recognizable in a crop, so early selection of seed-parents is quite easy. Often the primordia of staminate plants first appear as vegetative growth at the nodes along the main stalk and do not differentiate flowers for several weeks. Pistillate plants also may develop vegetative growth in place of the usual primordial calyxes and this growth makes staminate plants indistinguishable from pistillate plants for some time. This is often frustrating to sinsemilla Cannabis cultivators, since the staminate plants that are hesitant to differentiate sex take up valuable space that could be utilized by pistillate plants. Also, juvenile pistillate plants are occasionally mistaken for staminate plants if they are slow to form calyxes, since vegetative growth at the nodes could appear to be staminate primordia.

Latitude and Photoperiod

Change in photoperiod is the factor that usually triggers the developmental stages of Cannabis. Photoperiod and seasonal cycles are determined by latitude. The most even photoperiods and mildest seasonal variations are found near the equator, and the most widely fluctuating photoperiods and most radical seasonal variations are found in polar and high altitude locations. Areas in intermediate latitudes show more pronounced seasonal variation depending on their distance from the equator or height in altitude. A graph of light cycles based on latitude is

helpful in exploring the maturation and cycles of Cannabis from various latitudes and the genetic adaptations of strains to their native environments.

The wavy lines follow the changes in photoperiod (daylength) for two years at various latitudes. Follow, for example, the photoperiod for 400 north latitude (Northern California) which begins along the left-hand margin with a 15-hour photoperiod on June 21 (summer solstice). As the months progress to the right, the days get shorter and the line representing photoperiod slopes downward. During July the daylength decreases to 14 hours and Cannabis plants begin to flower and produce THC. (Increased THC production is represented by an increase in the size of the dots along the line of photoperiod.) As the days get shorter the plants flower more profusely and produce more THC until a peak period is reached during October and November. After this time the photoperiod drops below 10 hours and THC production slows. High-THC plants may continue to develop until the winter solstice (shortest day of the year, around December 21) if they are protected from frost. At this point a new vegetative light cycle starts and THC production ceases. New seedlings are planted when the days begin to get long (12-14 hours) and warm from March to May. Farther north at 600 latitude the day-length changes more radically and the growing season is shorter. These conditions do not favor THC production.

Light cycles and seasons vary as one approaches the equator. Near 200 north latitude (Hawaii, India, and Thailand where most of the finest drug Cannabis originates), the photoperiod never varies out of the range critical for THC production, between 10 and 14 hours. The light cycle at 200 north latitude starts at the summer solstice when the photoperiod is just a little over 13 hours. This means that a long season exists that starts earlier and finishes later than at higher latitudes. However, because the photoperiod is never too long to induce flowering, Cannabis may also be grown in a short season from December through March or April (90 to 120 days). Strains from these latitudes are often not as responsive to photoperiod change, and flowering seems strongly age-determined as well as light determined. Most strains of Cannabis will begin to flower when they are 60 days old if photoperiod does not exceed 13 hours. At 200 latitude, the photoperiod never exceeds 14 hours, and easily induced strains may begin flowering at nearly any time during the year.

Equatorial areas gain and lose daylength twice during the year as the sun passes north and south of the equator, resulting in two identical photoperiodic seasons. Rainfall snd altitude determine the growing season of each area, but at some locations along the equator it is possible to grow two crops of fully mature Cannabis in one year. By locating a particular latitude on the chart, and noting local dates for the last and first frosts and wet and dry seasons, the effective growing season may be determined. If an area has too short an effective growing season for drug Cannabis, a greenhouse or other shelter from cold, rainy conditions is used. The timing of planting and length of the growing season in these marginal conditions can also be determined from this chart.

For instance, assume a researcher wishes to grow a crop of Cannabis near Durban, South Africa, at 300 south latitude. Consulting the graph of maturation cycles will reveal that a long-photoperiod season, adequate for the maturation of drug Cannabis, exists from October through June. Local weather conditions indicate that average temperature ranges from 60~ to 80~ F. and annual precipitation from 30 to 50 inches. Early storms from the east in June could damage plants and some sort of storm protection might be necessary. Any estimates made from this chart sre generally accurate for photoperiod; however, local weather conditions are always taken into account.

Combination and simplification of the earth's climatic bands where Cannabis is grown yields an equatorial zone, north and south subtropical zones, north and south temperate zones, arctic and antarctic zones. A discussion of the maturation cycle for drug Cannabis in each zone follows.

Equatorial Zone - (15 south latitude to 15 north latitude)

At the equator the sun is high in the sky all year long. The sun is directly overhead twice a year at the equinoxes, March 22 and September 22, as it passes to the north and then the south. The days get shortest twice a year on each equinox. As a result, the equatorial zone has two times during the year when floral induction can take place and two distinct seasons, These seasons may overlap but they are usually five to six months long and unless the weather forbids, the fields may be used twice a year. Colombia, southern India, Thailand, and Malawi all lie on the fringes of the equatorial zone between 10 and 15 latitude. It is interesting to note that few if any areas of commercial Cannabis cultivation, other than Colombia, lie within the heart of the equatorial zone. This could be because most areas along the equator or very near to it are extremely humid at lower altitudes, so it may be impossible to find a dry enough place to grow one crop of Cannabis, much less two. Wild Cannabis occurs in many equatorial areas but it is of relatively low quality for fiber or drug production. Under cultivation, however, equatorial Cannabis has great potential for drug production.

Northern and Southern Subtropical Zones - (15 to 30 north and south latitudes)

The northern subtropical zone is one of the largest Cannabis producing areas in the world, while the southern subtropical zone has little Cannabis. These areas usually have a long season from February-March through October-December in the northern hemisphere and from September-October through March-June in the southern hemisphere. A short season may also exist from December or January through March or April in the northern hemisphere, spanning from 90 to 120 days. In Hawaii, Cannabis cultivators sometimes make use of a third short season from June through September or September through December, but these short seasons actually break up the long subtropical season during which some of the world's most potent Cannabis is grown. Southeast Asia, Hawaii,

Mexico, Jamaica, Pakistan, Nepal, and India are all major Cannabis-producing areas located in the northern subtropical zone.

North and South Temperate Zones - (30 to 60 north and south latitudes)

The temperate zones have one medium to long season stretching from March-May through September-December in the northern hemisphere and from September-November through March-June in the southern hemisphere. Central China, Korea, Japan, United States, southern Europe, Morocco, Turkey, Lebanon, Iran, Afghanistan, Pakistan, India, and Kashmir are all in the north temperate zone. Many of these nations are producers of large amounts of fiber as well as drug Cannabis. The south temperate zone includes only the southern portions of Australia, South America, and Africa. Some Cannabis grows in all three of these areas, but none of them are well known for the cultivation of drug Cannabis.

Arctic and Antarctic Zones - (60 to 70 north and south latitudes)

The arctic and antarctic zones are characterized by a short, harsh growing season that is not favorable for the growth of Cannabis, The arctic season begins during the very long days of June or July, as soon as the ground thaws, and continues until the first freezes of September or October. The photoperiod is very long when the seedlings appear, but the days rapidly get shorter and by September the plants begin to flower. Plants often get quite large in these areas, but they do not get a long enough season to mature completely and the cultivation of drug Cannabis is not practical without a greenhouse. Parts of Russia, Alaska, Canada, and northern Europe are within the arctic zone and only small stands of escaped fiber and drug Cannabis grow naturally. Cultivated drug strains are grown in Alaska, Canada, and northern Europe in limited quantities but little is grown on a commercial scale. Rapidly maturing, acclimatized hybrid strains from temperate North America are probably the best suited for growth in this area. Fiber strains also grow well in some arctic areas. Breeding programs with Russian Cannabis ruderalis could yield very short season drug strains.

It becomes readily apparent that most of the drug Cannabis occurs in the northern subtropical and northern temperate zones of the world. It is striking that there are many unutilized areas suitable for the cultivation of drug Cannabis the world over. It is also readily apparent that the equatorial zone and subtropical zones have the advantage of an extra full or partial season for the cultivation of Cannabis.

Strains that have become adapted to their native latitude will tend to flower and mature under domestic cultivation in much the same pattern as they would in their native conditions. For example, in northern temperate areas, strains from Mexico (subtropical zone) will usually completely mature by the end of October while strains from Colombia (equatorial zone) will usually not mature until

December. By understanding this, strains may be selected from latitudes similar to the area to be cultivated so that the chances of growing drug Cannabis to maturity are maximized. The short season of Hawaii, Mexico, and other subtropical areas constitutes a separate set of environmental factors (distinct from the long season) that influence genotype and favor selection of a separate short-season strain. The maturation characteristics can vary greatly between these two strains because of the length of the season and differences in response to photoperiod. For that reason, it is usually necessary to determine if Hawail and California strains have been bred specifically for either the short or long season, or if they are used indiscriminately for both seasons. Sometimes the only information available is what season the ~1 seed plant was grown. It may not be practical to grow a long-season strain from Hawaii in a temperate growing area, but a short season strain might do very well.

Moon Cycles

Since ancient times man has observed the effect of the moon on living organisms, especially his crops. Planting and harvest dates based on moon cycles are still found in the Old Farmer's Almanac. The moon takes 28 to 29 days to completely orbit the earth. This cycle is divided into four one-week phases. It starts as the new moon waxes (begins to enlarge) for a week until the quarter moon and another week until the moon is full. Then the waning (shrinking) cycle begins and the moon passes back for two weeks through another quarter to reach the beginning of the cycle with a new moon. Most cultivators agree that the best time for planting is on the waxing moon, and the best time to harvest is on the waning moon. Exact new moons, full moons, and quarter moons are avoided as these are times of interplanetary stress. Planting, germinating, grafting, and layering are most favored during phases 1 and 2. The best time is a few days before the full moon. Phases 3 and 4 are most beneficial for harvesting and pruning.

Root growth seems accelerated at the time of the new moon, possibly as a response to increased gravitational pull from the alignment of sun and moon. It also seems that floral cluster formation is slowed by the full moon. Strong, full moonlight is on the borderline of being enough light to cease floral induction entirely. Although this never happens, if a plant is just about to begin floral growth, it may be delayed a week by a few nights of bright moonlight.

Conversely, plants begin floral growth during the dark nights of the new moon. More research is needed to explain the mysterious effects of moon cycles on Cannabis

Floral Maturation

The individual pistillate calyxes and the composite floral clusters change as they mature. External changes indicate that internal biochemical metabolic changes

are also occurring. When the external changes can be connected with the invisible internal metabolic changes, then the cultivator is in a better position to decide when to har vest floral clusters. With years of experience this becomes intuition, but there are general correlations which can put the process in more objective terms.

The calyxes first appear as single, thin, tubular, green sheaths surrounding an ovule at the basal attached end with a pair of thin white, yellowish green, or purple pistils attached to the ovule and protruding from the tip fold of the calyx. As the flower begins to age and mature, the pistils grow longer and the calyx enlarges slightly to its full length. Next, the calyx begins to swell as resin secretion increases, and the pistils reach their peak of reproductive ripeness. From this point on, the pistils begin to swell and darken slightly, and the tips may begin to curl and turn reddish brown. At this stage the pistillate flower is past its reproductive peak, and it is not likely that it will produce a viable seed if pollinated. Without pollination the calyx begins to swell almost as if it had been fertilized and resin secretion reaches a peak. The pistils eventually wither and turn a reddish or orange brown. By this time, the swollen calyx has accumulated an incredible layer of resin, but secretion has slowed and few fresh terpenes and cannabinoids are being produced. Falling pistils mark the end of the developmental cycle of the individual pistillate calyx. The resins turn opaque and the calyx begins to die.

The biosynthesis of cannabinoids and terpenes parallels the developmental stages of the calyx and associated resin-producing glandular trichomes. Also, the average developmental stage of the accumulated individual calyxes determines the maturational state of the entire floral cluster. Thus, determination of maturational stage and timing of the harvest is based on the average calyx and resin condition, along with general trends in morphology and development of the plant as a whole.

The basic morphological characteristics of floral maturation are measured by calyx-to-leaf ratio and internode length within floral clusters. Calyx-to-leaf ratios are highest during the peak floral stage. Later stages are usually characterized by decreased calyx growth and increased leaf growth. Internode length is usually very short between pairs of calyxes in tight dense clusters. At the end of the maturation cycle, if there is still growth, the internode length may increase in response to increased humidity and lowered light conditions. This is most often a sign that the floral clusters are past their reproductive peak; if so, they are preparing for rejuvenation and the possibility of re-growth the following season. At this time nearly all resin secretion has ceased at temperate latitudes (due to low temperatures), but may still continue in equatorial and subtropical areas that have a longer and warmer growing season. Greenhouses have been used in temperate latitudes to simulate tropical environments and extend the period of resin production. It should be remembered that greenhouses also tend to cause

a stretched condition in the floral clusters in response to high humidity, high temperatures, lowered light intensity, and restricted air circulation.

Simulation of the native photoperiod of a certain strain is achieved through the use of blackout curtains and supplemental lighting in a greenhouse or indoor environment. The localized light cycle particular to a strain may be estimated from the graph of maturation patterns at various latitudes (p.124). In this way it is possible to reproduce exotic foreign environments to more accurately study Cannabis, Tight clusters of calyxes and leaves are characteristic of ripe outdoor Cannabis. Some strains, however, such as those from Thailand, tend to have longer internodes and appear airy and stretched. This seems to be a genetically controlled adaptation to their native environment. Imported examples from Thailand also have long internodes in the pistillate floral clusters. Thai strains may not develop tight floral clusters even in the most arid and exposed conditions; however, this condition is furthered as rejuvenation begins during autumn days of decreasing photoperiod.

Cannabinoid Biosynthesis

Since resin secretion and associated terpenoid and cannabinoid biosynthesis are at their peak just after the pistils have begun to turn brown but before the calyx stops growing, it seems obvious that floral clusters should be harvested during this time. More subtle variations in terpenoid and cannabinoid levels also take place within this period of maximum resin secretion, and these variations influence the nature of the resin’s psychoactive effect.

The cannabinoid ratios characteristic of a strain are primarily determined by genes, but it must be remembered that many environmental factors, such as light, temperature, and humidity, influence the path of a molecule along the cannabinoid biosynthetic pathway. These environmental factors can cause an atypical final cannabinoid profile (cannabinoid levels and ratios). Not all cannabinoid molecules begin their journey through the pathway at the same time, nor do all of them complete the cycle and turn into THC molecules simultaneously. There is no magical way to influence the cannabinoid biosynthesis to favor THC production, but certain factors involved in the growth and maturation of Cannabis do affect final cannabinoid levels, These factors may be controlled to some extent by proper selection of mature floral clusters for harvesting, agricul tural technique, and local environment. In addition to genetic and seasonal influences, the picture is further modified by the fact that each individual calyx goes through the cannabinoid cycle fairly independently and that during peak periods of resin secretion new flowers are produced every day and begin their own cycle. This means that at any given time the ratio of calyx-to-leaf, the average calyx condition, the condition of the resins, and resultant cannabinoid ratios indicate which stage the floral cluster has reached. Since it is difficult for the amateur cultivator to determine the cannabinoid profile of a floral cluster without chromatographic analysis, this discussion will center on the

known and theoretical correlations between the external characteristics of calyx and resin and internal cannabinoid profile. A better understanding of these subtle changes in cannabinoid ratios may be gleaned by observing the cannabinoid biosynthesis. Focus on the lower left-hand corner of the chart. Next, follow the chain of reactions until you find the four isomers of THC acid (tetrahydro-cannabinolic acid), toward the right side of the page at the crest of the reaction sequence, and realize that there are several steps in a long series of reactions that precede and follow the formation of THC acids, the major psychoactive cannabinoids. Actually, THC acid and the other necessary cannabinoid acids are not psychoactive until they decarboxylate (lose an acidic carboxyl group [COOHI). It is the cannabinoid acids which move along the biosynthetic pathway, and these acids undergo the strategic reactions that determine the position of any particular cannabinoid molecule along the pathway. After the resins are secreted by the glandular trichome they begin to harden and the cannabinoid acids begin to decarboxylate. Any remaining cannabinoid acids are decarboxylated by heat within a few days after harvesting. Other THC acids with shorter side-chains also occur in certain strains of Cannabis. Several are known to be psychoactive and many more are suspected of psychoactivity. The shorter propyl (three-carb on) and methyl (one-carbon) side-chain homologs (similarly shaped molecules) are shorter acting than pen tyl (five-carbon) THCs and may account for some of the quick, flashy effects noted by some marijuana users. We will focus on the pentyl pathway but it should be noted that the propyl and methyl pathways have homologs at nearly every step along the pentyl pathway and their synthesis is basically identical.

The first step in the pentyl cannabinoid biosynthetic pathway is the combination of olivetolic acid with geranyl pyrophosphate. Both of these molecules are derived from terpenes, and it is readily apparent that the biosynthetic route of the aromatic terpenoids may be a clue to formation of the cannabinoids. The union of these two molecules forms CBG acid (cannabigerolic acid) which is the basic cannabinoid precursor molecule. CBG acid may be converted to CBGM (CBG acid monomethyl ether), or a hydroxyl group (OH) attaches to the geraniol portion of the molecule forming hydroxy-CBG acid. Through the formation of a transition-state molecule, either CBC acid (cannabichromenic acid) or CBD acid (cannabidiolic acid) is formed. CBD acid is the precursor to the THC acids, and, although CBD is only mildly psychoactive by itself, it may act with THC to modify the psychoactive effect of the THC in a sedative way. CBC is also mildly psychoactive and may interact synergistically with THC to alter the psychoactive effect (Turner et al. 1975). Indeed, CBD may suppress the effect of THC and CBC may potentiate the effect of THC, although this has not yet been proven. All of the reactions along the cannabinoid biosynthetic pathway are enzyme-controlled but are affected by environmental conditions.

Conversion of CBD acid to THC acid is the single most important reaction with respect to psychoactivity in the entire pathway and the one about which we know the most. Personal communication with Raphael Mechoulam has centered

around the role of ultraviolet light in the bio-synthesis of THC acids and minor cannabinoids. In the laboratory, Mechoulam has converted CBD acid to THC acids by exposing a solution of CBD acid in n-hexane to ultraviolet light of 235-285 nm. for up to 48 hours. This reaction uses atmospheric oxygen molecules (02) and is irreversible; however, the yield of the conversion is only about 15% THC acid, and some of the products formed in the laboratory experiment do not occur in living specimens. Four types of isomers or slight variations of THC acids (THCA) exist. Both Delta1-THCA and Delta6-THCA are naturally occurring isomers of THCA resulting from the positions of the double bond on carbon 1 or carbon 6 of the geraniol portion of the molecule They have approximately the same psychoactive effect; however, Delta1-THC acid is about four times more prevalent than Delta6-THC acid in most strains. Also Alpha and Beta forms of Delta1-THC acid and Delta6-THC acid exist as a result of the juxtaposition of the hydrogen (H) and the carboxyl (COOH) groups on the olivetolic acid portion of the molecule It is suspected that the psychoactivity of the a and ~ forms of the THC acid molecules probably does not vary, but this has not been proven. Subtle differences in psychoactivity not detected in animals by laboratory instruments, but often discussed by marijuana aficionados, could be attributed to additional synergistic effects of the four isomers of THC acid. Total psycho-activity is attributed to the ratios of the primary cannabinoids of CBC, CBD, THC and CBN; the ratios of methyl, propyl, and pentyl homologs of these cannabinoids; and the isomeric variations of each of these cannabinoids. Myriad subtle combinations are sure to exist. Also, terpenoid and other aromatic compounds might suppress or potentiate the effects of THCs.

Environmental conditions influence cannabinoid biosynthesis by modifying enzymatic systems and the resultant potency of Cannabis. High altitude environments are often more arid and exposed to more intense sunlight than lower environments. Recent studies by Mobarak et al. (1978) of Cannabis grown in Afghanistan at 1,300 meters (4,350 feet) elevation show that significantly more propyl cannabinoids are formed than the respective pentyl homo-logs. Other strains from this area of Asia have also exhibited the presence of propyl cannabinoids, but it cannot be discounted that altitude might influence which path of cannabinoid biosynthesis is favored. Aridity favors resin production and total cannabinoid production; however, it is unknown whether arid conditions promote THC production specifically. It is suspected that increased ultraviolet radiation might affect cannabinoid production directly. Ultra-violet light participates in the biosynthesis of THC acids from CBD acids, the conversion of CBC acids to CCY acids, and the conversion of CBD acids to CBS acids. However, it is unknown whether increased ultraviolet light might shift cannabinoid synthesis from pentyl to propyl pathways or influence the production of THC acid or CBC acid instead of CBD acid.

The ratio of THC to CBD has been used in chemotype determination by Small and others. The genetically determined inability of certain strains to convert CBD acid to THC acid makes them a member of a fiber chemotype, but if a strain has

the genetically determined ability to convert CBD acid to THC acid then it is considered a drug strain. It is also interesting to note that Turner and Hadley (1973) discovered an African strain with a very high THC level and no CBD although there are fair amounts of CBC acid present in the strain. Turner* states that he has seen several strains totally devoid of CBD, but he has never seen a strain totally devoid of THC. Also, many early authors confused CBC with CBD in analyzed samples because of the proximity of their peaks on gas liquid chromatograph (GLC) results. If the biosynthetic pathway needs alteration to include an enzymatically controlled system involving the direct conversion of hydroxy-CBG acid to THC acid through allylic rearrangement of hydroxy-CBG acid and cyclization of the rearranged intermediate to THC acid, as Turner and Hadley (1973) suggest, then CBD acid would be bypassed in the cycle and its absence explained. Another possibility is that, since CBC acid is formed from the same symmetric intermediate that is allylically rearranged before forming CBD acid, CBC acid may be the accumulated intermediate, the reaction may be reversed, and through the symmetric intermediate and the usual allylic rearrangement CBD acid would be formed but directly converted to THC acid by a similar enzyme system to that which reversed the formation of CBC acid. If this happened fast enough no CBD acid would be detected. It is more likely, however, that CBDA in drug strains is converted directly to THCA as soon as it is formed and no CBD builds up. Also Turner, Hemphill, and Mahlberg (1978) found that CBC acid was contained in the tissues of Cannabis but not in the resin secreted by the glandular trichomes. In any event, these possible deviations from the accepted biosynthetic pathway provide food for thought when trying to decipher the mysteries of Cannabis strains and varieties of psychoactive effect.

Returning to the more orthodox version of the cannabinoid biosynthesis, the role of ultraviolet light should be reemphasized. It seems apparent that ultraviolet light, normally supplied in abundance by sunlight, takes part in the conversion of CBD acid to THC acids. Therefore, the lack *Carlton Thrner 1979: personal communication. of ultraviolet light in indoor growing situations could account for the limited psychoactivity of Cannabis grown under artificial lights. Light energy has been collected and utilized by the plant in a long series of reactions resulting in the formation of THC acids. Farther along the pathway begins the formation of degradation products not metabolically produced by the living plant. These cannabinoid acids are formed through the progressive degradation of THC acids to CBN acid (cannabinolic acid) and other cannabinoid acids. The degradation is accomplished primarily by heat and light and is not enzymatically controlled by the plant. CBN is also suspected of synergistic modification of the psychoactivity of the primary cannabinoids, THCs. The cannabinoid balance between CBC, CBD, THC, and CBN is determined by genetics and maturation. THC production is an ongoing process as long as the glandular trichome remains active. Variations in the level of THC in the same trichome as it matures are the result of THC acid being broken down to CBN acid while CBD acid is being converted to THC acid. If the rate of THC biosynthesis exceeds the rate of THC breakdown, the THC level in the trichome rises; if the breakdown rate is faster than the rate of

biosynthesis, the THC level drops. Clear or slightly amber transparent resin is a sign that the glandular trichome is still active. As soon as resin secretion begins to slow, the resins will usually polymerize and harden. During the late floral stages the resin tends to darken to a transparent amber color. If it begins to deteriorate, it first turns translucent and then opaque brown or white. Near-freezing temperatures during maturation will often result in opaque white resins. During active secretion, THC acids are constantly being formed from CBD acid and breaking down into CBN acid.

Harvest Timing

With this dynamic picture of the biosynthesis and degradation of THC acids as a frame of reference, the logic behind harvesting at a specific time is easier to understand. The usual aim of timing the moment of harvest is to ensure high THC levels modified by just the proper amounts of CBC, CBD and CBN, along with their propyl homologs, to approximate the desired psychoactive effect. Since THC acids are being broken down into CBN acid at the same time they are being made from CBD acid, it is important to harvest at a time when the production of THC acids is higher than the degradation of THC acids. Every experienced cultivator inspects a number of indicating factors and knows when to harvest the desired type of floral clus ters. Some like to harvest early when most of the pistils are still viable and at the height of reproductive potential. At this time the resins are very aromatic and light; the psychoactive effect is characterized as a light cerebral high (possibly low CBC and CBD, high THC, low CBN). Others harvest as late as possible, desiring a stronger, more resinous marijuana characterized by a more intense body effect and an inhibited cerebral effect (high CBC and CB]), high THC, high CBN). Harvesting and testing several floral clusters every few days over a period of several weeks gives the cultivator a set of samples at all stages of maturation and creates a basis for deciding when to harvest in future seasons. The following is a description of each of the growth phases as to morphology, terpene aroma, and relative psychoactivity.

Premature Floral Stage

At this stage floral development is slightly beyond primordial and only a few clusters of immature pistillate flowers appear at the tips of limbs in addition to the primordial pairs along the main stems. By this stage stem diameter within the floral clusters is very nearly maximum. The stems are easily visible between the nodes and form a strong framework to support future floral development. Larger vegetative leaves (5-7 leaflets) predominate and smaller tri-leaflet leaves are beginning to form in the new floral axis. A few narrow, tapered calyxes may be found nestled in the leaflets near the stem tips and the fresh pistils appear as thin, feathery, white filaments stretching to test the surroundings. During this stage the surface of the calyxes is lightly covered with fuzzy, hair-like, non-glandular trichomes, but only a few bulbous and capitate-sessile glandular trichomes have begun to develop. Resin secretion is minimal, as indicated by

small resin heads and few if any capitate-stalked, glandular trichomes. There is no drug yield from plants at the premature stage since THC production is low, and there is no economic value other than fiber and leaf. Terpene production starts as the glandular trichomes begin to secrete resin; premature floral clusters have no terpene aromas or tastes. Total cannabinoid production is low but simple cannabinoid phenotypes, based on relative amounts of THC and CBD, may be determined. By the pre-floral stage the plant has akeady established its basic chemotype as a fiber or drug strain. A fiber strain rarely produces more than 2% THC, even under perfect agricultural conditions. This indicates that a strain either produces some varying amount of THC (up to 13%) and little CBD and is termed a drug strain or produces practically no THC and high CBD and is termed a fiber strain, This is genetically controlled.

The floral clusters are barely psychoactive at this stage, and most marijuana smokers classify the reaction as more an "effect" than a "high." This most likely results from small amounts of THC as well as trace amounts of CBC and CBD. CBD production begins when the seedling is very small. THC production also begins when the seedling is very small, if the plant originates from a drug strain. However, THC levels rarely exceed 2% until the early floral stage and rarely produce a "high" until the peak floral stage.

Early Floral Stage

Floral clusters begin to form as calyx production increases and internode length decreases. Tri-leaflet leaves are the predominant type and usually appear along the secondary floral stems within the individual clusters. Many pairs of calyxes appear along each secondary floral axis and each pair is subtended by a tri-leaflet leaf. Older pairs of calyxes visible along the primary floral axis during the premature stage now begin to swell, the pistils darken as they lose fertility, and some resin secretion is observed in trichomes along the veins of the calyx. The newly produced calyxes show few if any capitate-stalked trichomes. As a result of low resin production, only a slight terpene aroma and psychoactivity are detectable. The floral clusters are not ready for harvest at this point. Total cannabinoid production has increased markedly over the premature stage but THC levels (still less than 3%) are not high enough to produce more than a subtle effect.

Peak Floral Stage

Elongation growth of the main floral stem ceases at this stage, and floral clusters gain most of their size through the addition of more calyxes along the secondary stems until they cover the primary stem tips in an overlapping spiral. Small reduced mono-leaflet and tri-leaflet leaves subtend each pair of calyxes emerging from secondary stems within the floral clusters. These subtending leaves are correctly referred to as bracts. Outer leaves begin to wilt and turn yellow as the pistillate plant reaches its reproductive peak. In the primordial

calyxes the pistils have turned brown; however, all but the oldest of the flowers are fertile and the floral clusters are white with many pairs of ripe pistils. Resin secretion is quite advanced in some of the older infertile calyxes, and the young pistillate calyxes are rapidly producing capitate-stalked glandular trichomes to protect the precious unfertilized ovule. Under wild conditions the pistillate plant would be starting to form seeds and the cycle would be drawing to a close. When Cannabis is grown for sinsemilla floral production, the cycle is interrupted. Pistillate plants remain unfertilized and begin to produce capitate -stalked trichomes and accumulate resins in a last effort to remain viable. Since capitate-stalked trichomes now predominate, resin and THC production increase. The elevated resin heads appear clear, since fresh resin is still being secreted, often being produced in the cellular head of the trichome. At this time THC acid production is at a peak and CBD acid levels remain stable as the molecules are rapidly converted to THC acids, THC acid synthesis has not been active long enough for a high level of CBN acid to build up from the degradation of THC acid by light and heat. Terpene production is also nearing a peak and the floral clusters are beautifully aromatic. Many cultivators prefer to pick some of their strains during this stage in order to produce marijuana with a clear, cerebral, psychoactive effect. It is believed that, in peak floral clusters, the low levels of CBD and CBN allow the high level of THC to act without their sedative effects. Also, little polymerization of resins has occurred, so aromas and tastes are often less resinous and tar like than at later stages. Many strains, if they are harvested in the peak floral stage, lack the completely developed aroma, taste and psychoactive level that appear after curing. Cultivators wait longer for the resins to mature if a different taste and psychoactive effect is desired.

This is the point of optimum harvest for some strains, since most additional calyx growth has ceased. However, a subsequent flush of new calyx growth may occur and the plant continue ripening into the late floral stage.

Late Floral Stage

By this stage plants are well past the main reproductive phase and their health has begun to decline. Many of the larger leaves have dropped off, and some of the small inner leaves begin to change color. Autumn colors (purple, orange, yellow, etc.) begin to appear in the older leaves and calyxes at this time; many of the pistils turn brown and begin to fall off. Only the last terminal pistils are still fertile and swollen calyxes predominate. Heavy layers of protec tive resin heads cover the calyxes and associated leaves. Production of additional capitate-stalked glandular trichomes is rare, although some existing trichomes may still be elongating and secreting resins. As the previously secreted resins mature, they change color. The polymerization of small terpene molecules (which make up most of the resin) produces long chains and a more viscous and darker-colored resin. The ripening and darkening of resins follows the peak of psychoactive cannabinoid synthesis and the transparent amber color of mature resin is usually indicative of high THC content. Many cultivators agree that transparent amber

resins are a sign of high-quality drug Cannabis and many of the finest strains exhibit this characteristic. Particularly potent Cannabis from California, Hawaii, Thailand, Mexico, and Colombia is often encrusted with transparent amber colored instead of clear resin heads. This is also characteristic of Cannabis from other equatorial, subtropical and temperate zones where the growing season is long enough to accommodate long term resin production and maturation. Many areas of North America and Europe have too short a season to fully mature resins unless a greenhouse is used. Specially acclimatized strains are another possibility. They develop rapidly and begin maturing in time to ripen amber resins while the weather is still warm and dry.

The weight yield of floral clusters is usually highest at this point, but strains may begin to grow an excess of leaves in late-stage clusters to catch additional energy from the rapidly diminishing autumn sun. Total resin accumulation is highest at this stage, but the period of maximum resin production has passed. If climatic conditions are harsh, resins and cannabinoids will begin to decompose. As a result, resin yield may appear high even if many of the resin heads are missing or have begun to deteriorate and the overall psychoactivity of the resin has dropped. THC decomposes to CBN in the hot sun and will not remain intact or be replaced after the metabolic processes of the plant have ceased. Since cannabinoids are so sensitive to decomposition by sunlight, the higher psychoactivity of amber resins may be a secondary effect. It may be that the THC is better protected from the sun by amber or opaque resins than by clear resins. Some late maturing strains develop opaque, white resin heads as a result of terpene polymerization and THC decomposition. Opaque resin heads are usually a sign that the floral clusters are over-mature.

Late floral clusters exhibit the full potential of resin production, aromatic principles, and psychoactive effect. Complex mixtures of many mon oterpene and sesquiterpene hydrocarbons along with alcohols, ethers, esters, and ketones determine the aroma and flavor of mature Cannabis. The levels of the basic terpenes and their polymerized by-products fluctuate as the resin ripens. The aromas of fresh floral clusters are usually preserved after drying, as by the late floral stage, a high proportion of ripe resins are present on the mature calyxes of the fresh plant. Cannabinoid production favors high THC acid and rising CBN acid content at this stage, since most active biosynthesis has ceased and more THC acid is being broken down into CBN acid than is being produced from CBD acid. CBD acid may accumulate because not enough energy is available to complete its conversion to THC acid. The THC-to-CBD ratio in the harvested floral clusters certainly begins to drop as biosynthesis slows, because THC acid levels decrease as it decom poses, and at the same time CBD acid levels remain or rise intact since CBD does not decompose as rapidly as THC acid. This tends to produce marijuana characterized by more somatic and sedative effects. Some cultivators prefer this to the more cerebral and clear psychoactivity of the peak floral stage.

Senescence or Rejuvenation Stage

After a pistillate plant finishes floral maturation, the production of pistillate calyxes ceases and the plant continues senescence (decline towards death). In unusual situations, however, rejuvenation will begin and the plant will sprout new vegetative growth in preparation for the following season. Senescence is often highlighted by striking color changes in the floral clusters. Leaves, calyxes, and stems display auxiliary pigments ranging in color from yellow through red to deep purple. Eventually a brown shade pre-dominates and death is near. In warm areas, rejuvenation starts as vegetative shoots form within the floral clusters. These shoots are usually made up of unserrated single leaflets separated by thin stems with long internodes. It is as if the plant were reaching for limited winter light. Leaf production is accelerated as plants reach the rejuvenation stage, and resin production completely stopped. Floral clusters left to ripen until the bitter end usually produce inferior marijuana of lowered THC level, especially outdoors in bad weather.

Terpene secretion changes along with cannabinoid secretion and psychoactive effect. Various terpenes, terpene polymers, and other aromatic principles are produced and ripen at different times in the development of the plant. If these changes in aromatic principles are directly correlated with changes in cannabinoid production, then harvest selections for cannabinoid level may be possible based on the aroma of the ripening floral clusters.

It is important to understand differences in the anatomy of floral clusters for each Cannabis strain. Trends in the relative quantity (dry weight) of various parts (such as leaves, calyxes and trichomes) at various harvest dates are characteristic of particular strains and may vary widely. Some generalizations can be made. In most cases, the percentage of stem weight steadily decreases as the floral cluster matures. Rejuvenation growth can account for a sudden increase in stem percentage. The percentage of inner leaves usually starts very low and climbs rapidly as the floral clus ters mature. This often reflects increased leaf growth near the end of the season. In many strains the percentage of inner leaves drops sharply during the peak floral stage and rises again as calyx production slows and leaf production increases in the late floral stage.

Calyx production follows two basic patterns. In one, the percentage of calyxes climbs gradually and levels out during the peak floral stage. It begins to decline in the late floral stage, and leaf production increases as calyx production ceases. Other strains continue to produce calyxes at the expense of leaves, and the calyx percentage increases steadily throughout maturation. In both cases, there is some tendency for calyx percentage to level out during the peak floral stage irrespective of whether leaf growth accelerates or calyx growth continues at a later stage.

Resins generally accumulate steadily while the plant matures, but strains may vary as to the stage of peak resin secretion. Seed percentage increases exponentially with time if the crop is well fertilized, but most samples of drug Cannabis grown domestically are nearly seedless.

To determine dry weight, samples are harvested, labeled, and air dried until the central stem of the floral cluster will snap when bent. In plant research, dry weight is done in ovens at higher temperatures, but these higher temperatures would ruin the Cannabis. The dry floral cluster is weighed. The outer leaves, inner leaves, calyxes, seeds, and stems are segregated and each group weighed individually. The percentage is determined by dividing the individual dry weights by the total dry weight.

Calyx percentage ranges from 30 to 70% of the dry weight of the seedless floral clusters, depending on variety and harvest date. Inner leaf percentages fluctuate between 15 and 45% of dry weight; stems range from 10 to 30%. It seems obvious that for drug harvesting a maximum calyx production is important to quality resin production. A strain where maximum calyx production occurs simultaneously with peak resin production is a breeding goal not yet attained.

Harvesting Cannabis at the proper time requires information on how floral clusters mature and a decision on the part of the cultivator as to what type of floral clusters are desired. With harvesting as with other techniques of cultivation, the path to success is straightened when a definite goal is established. Personal preference is always the ultimate deciding factor.

Factors Influencing THC Production

Many factors influence the production of THC. In general, the older a plant, the greater its potential to produce THC. This is true, however, only if the plant remains healthy and vigorous, THC production requires the proper quantity and quality of light. It seems that none of the biosynthetic processes operate efficiently when low light conditions prevent proper photosynthesis. Research has shown (Valle et al. 1978) that twice as much THC is produced under a 12-hour photoperiod than under a 10-hour photoperiod. Warm temperatures are known to promote metabolic activity and the production of THC. Heat also promotes resin secretion, possibly in response to the threat of floral desiccation by the hot sun, Resin collects in the heads of glandular trichomes and does not directly seal the pores of the calyx to prevent desiccation. Resin heads may serve to break up the rays of the sun so that fewer of them strike the leaf surface and raise the temperature. However, light and heat also destroy THC. In a drug strain, a bio-synthetic rate must be maintained such that substantially more THC is produced than is broken down. Humidity is an interesting parameter of THC production and one of the least understood. Most high-quality drug Cannabis grows in areas that

are dry much of the time at least during the maturation period. It follows that increased resin produc. tion in response to arid conditions might account for increased THC production. High-THC strains, however, also grow in very humid conditions (greenhouses and equatorial zones) and produce copious quantities of resin. Cannabis seems not to produce more resins in response to dry soil, as it does to a dry atmosphere. Drying out plants by with-holding water for the last weeks of flowering does not stimulate THC production, although an arid atmosphere may do so. A Cannabis plant in flower requires water, so that nutrients are available. for operating the various bio-synthetic pathways.

There is really no confirmed method of forcing increased THC production. Many techniques have developed through misinterpretations of ancient tradition. In Colombia, farmers girdle the stalk of the main stem, which cuts off the flow of water and nutrients between the roots and the shoots. This technique may not raise the final THC level, but it does cause rapid maturation and yellow gold coloration in the floral cluster (Partridge 1973). Impaling with nails, pine splinters, balls of opium, and stones are clandestine folk methods of promoting flowering, taste and THC production. However none of these have any valid documentation from the original culture or scientific basis. Symbiotic relationships between herbs in companion plantings are known to influence the production of essential oils. Experiments might be carried out with different herbs, such as stinging nettles, as companion plants for Cannabis, in an effort to stimulate resin production. In the future, agricultural techniques may be discovered which specifically promote THC biosynthesis.

In general, it is considered most important that the plant be healthy for it to produce high THC levels. The genotype of the plant, a result of seed selection, is the primary factor which determines the THC levels. After that, the provision of adequate organic nutrients, water, sunlight, fresh air, growing space, and time for maturation seems to be the key to producing high-THC Cannabis in all circumstances. Stress resulting from inadequacies in the environment limits the true expression of phenotype and cannabinoid potential. Cannabis finds a normal adaptive defense in the production of THC laden resins, and it seems logical that a healthy plant is best able to raise this defense. Forcing plants to produce is a perverse ideal and alien to the principles of organic agriculture. Plants are not machines that can be worked faster and harder to produce more. The life processes of the plant rely on delicate natural balances aimed at the ultimate survival of the plant until it reproduces. The most a Cannabis cultivator or researcher can expect to do is provide all the requisites for healthy growth and guide the plant until it matures.

Flowering in Cannabis may be forced or accelerated by many different techniques. This does not mean that THC production is forced, only that the time before and during flowering is shortened and flowers are produced rapidly. Most techniques involve the deprivation of light during the long days of summer to promote early floral induction and sexual differentiation. This is sometimes done

by moving the plants inside a completely dark structure for 12 hours of each 24-hour day until the floral clusters are mature. This stimulates an autumn light cycle and promotes flowering at any time of the year. In the field, covers may be made to block out the sun for a few hours at sunrise or sunset, and these are used to cover small plants. Photoperiod alteration is most easily accomplished in a greenhouse, where blackout curtains are easily rolled over the plants. Drug Cannabis production requires 11-12 hours of continuous darkness to induce flowering and at least 10 hours of light for adequate THC production (Valle et al. 1978). In a greenhouse, supplemental lighting need be used only to extend daylength, while the sun supplies the energy needed for growth and THC biosynthesis. It is not known why at least 10 hours (and preferably 12 or 13 hours) of light are needed for high THC production. This is not dependent on accumulated solar energy since light responses can be activated and THC production increased with only a 40-watt bulb. A reasonable theory is that a light-sensitive pigment in the plant (possibly phytochrome) acts as a switch, causing the plant to follow the flowering cycle. THC production is probably associated with the induction of flowering resulting from the photoperiod change.

Cool night temperatures seem to promote flowering in plants that have previously differentiated sexually. Extended cold periods, however, cause metabolic processes to slow and maturation to cease. Most temperate Cannabis strains are sensitive to many of the signs of an approaching fall season and respond by beginning to flower. In contrast, strains from tropical areas, such as Thailand, often seem unresponsive to any signs of fall and never speed up development.

Contrary to popular thought, planting Cannabis strains later in the season in temperate latitudes may actually promote earlier flowering. Most cultivators believe that planting early gives the plant plenty of time to flower and it will finish earlier. This is often not true. Seedlings started in February or March grow for 4-5 months of increasing photoperiod before the days begin to get shorter following the solstice in June. Huge vegetative plants grow and may form floral inhibitors during the months of long photo-period. When the days begin to get shorter, these older plants may be reluctant to flower because of the floral inhibitors formed in the pre-floral leaves. Since floral cluster formation takes 6-10 weeks, the initial delay in flowering could push the harvest date into November or December. Cannabis started during the short days of December or January will often differentiate sex by March or April. Usually these plants form few floral clusters and rejuvenate for the long season ahead. No increased potency has been noticed in old rejuvenated plants. Plants started in late June or early July, after the summer solstice, are exposed only to days of decreasing photoperiod. When old enough they begin flowering immediately, possibly because they haven’t built up as many long-day floral inhibitors. They begin the 6-10 week floral period with plenty of time to finish during the warmer days of October. These later plantings yield smaller plants because they have a shorter vegetative cycle. This may prove an advantage. in greenhouse research, where it is common for plants to grow far too large for easy handling before they begin to

flower. Late plantings after the summer solstice receive short inductive photoperiods almost immediately. However, flowering is delayed into September since the plant must grow before it is old enough to flower. Although flowering is delayed, the small plants rapidly produce copious quantities of flowers in a final effort to reproduce.

Extremes in nutrient concentrations are considered influential in both the sex determination and floral development of Cannabis. High nitrogen levels in the soil during the seedling stage seem to favor pistillate plants, but high nitrogen levels during flowering often result in delayed maturation and excessive leafing in the floral clusters. Phosphorus and potassium are both vital to the floral maturation of Cannabis. High-phosphorus fertilizers known as "bloom boosters" are available, and these have been shown to accelerate flowering in some plants. However, Cannabis plants are easily burned with high phosphorus fertilizers since they are usually very acidic. A safer method for the plant is the use of natural phosphorus sources, such as colloidal phosphate, rock phosphate, or bone meal; these tend to cause less shock in the maturing plant. They are a source of phosphorus that is readily available as well as long-term in effect. Chemical fertilizers sometimes produce floral clusters with a metallic, salty flavor. Extremes in nutrient levels usually affect the growth of the entire plant in an adverse way.

Hormones, such as gibberellic acid, ethylene, cytokinins and auxins, are readily available and can produce some strange effects. They can stimulate flowering in some cases, but they also stimulate sex reversal. Plant physiology is not simple, and results are usually unpredictable.

Harvesting, Drying, and Curing

Cannabis is cultivated for the harvest of several different commercial products. Pulp, fiber, seed, drugs, and resin are produced from various parts of the Cannabis plant. The methods of harvesting, drying, curing, and storing various plant parts are determined by the intended use of the plant. Pulp is made from the leaves of juvenile plants and from waste products of fiber and drug production. Fibers are produced from the stems of the Cannabis plant. The floral clusters are responsible for the production of seeds, drugs, and aromatic resins.

If plants are to be used solely as a pulp source for paper production, they may be harvested at any point in the life cycle when they are large enough to produce a reasonable yield of leaves and small stems. The leaves and small stems are stripped from the larger stalks, and after drying they are bailed and stored or made directly into paper pulp. Cannabis contains approximately 67% cellulose and 16% hemicellulose; this makes a fine resilient paper. In Italy, the finest Bibles are printed on hemp paper.

Fiber or hemp Cannabis is usually grown in large, crowded fields. Crowding of seedlings results in tall, thin plants with few limbs and long, straight fibers. The

total field is harvested when the fiber content reaches the correct level but before the fibers begin to lignify or harden. The cut stalks are stripped of leaves and bundled to dry. Fibers are extracted by natural or chemical retting, Retting is the breaking down of the outside skin layer and tissues that join the fibers into bundles, so that the individual fibers are freed. Natural retting is accomplished by soaking the stalks in water and laying them out on the ground, where they are attacked by decay organisms such as fungi and bacteria. Dew may also wet the stalks, and they are turned frequently to evenly wet them and avoid excessive decay. Continued soaking, attack by organisms, and pounding of the stalks results in the liberation of individual fibers from their vascular bundles. Natural retting takes from one week to a month. The fibers are thoroughly dried, wrapped in bundles and stored in a cool, dry area. The yield of fiber is approximately 25% of the weight of the dried stalks.

Seeds are harvested by cutting fields of seeded pistillate plants and removing the seeds either by hand or machine. Cannabis seeds usually fall easily from the floral clusters when mature. The remainder of the plant may be used as pulp material or low-grade marijuana. The Indian tradition of preparing ganja is by walking on it and rolling it between the palms to remove excess seeds and leaves.

Seeds are allowed to dry completely and all vegetable debris is removed before storage. This prevents spoilage caused by molds and other fungi. Seeds to be used for oil production may be stored in bags, boxes, or jars, and not exposed to excess humidity (causing them to germinate) or excessive aridity (causing them to dry out and crack). Seeds preserved for future germination are thoroughly air dried in paper envelopes or cloth sacks and stored in air-tight containers in a cool, dark, dry place. Freezing may also dry out seeds and cause them to crack. If seeds are carefully stored, they remain viable for a number of years. As a batch of seeds ages, fewer and fewer of them will germinate, but even after 5 to 6 years a small percentage of the seeds usually still germinate. Old batches of seeds also tend to germinate slowly (up to 5 weeks). This means that a batch of seeds for cultivation might be stored for a longer time if the initial sample is large enough to provide sufficient seeds for another generation. If a strain is to be preserved, it is necessary to grow and reproduce it every three years, so that enough viable seeds are always available.

Curing Floral Clusters

Harvesting, drying, curing, and storage of Cannabis floral clusters to preserve and enhance appearance, taste, and psychoactivity is often discussed among cultivators. More floral clusters are ruined by poor handling after harvest than by any other single cause. When the plant is harvested, the production of fine floral clusters for smoking begins. Cannabis floral clusters are harvested by two basic methods: either individually, by cutting them from the stalks and carefully packaging them in shallow boxes or trays, or all simultaneously by uprooting or

cutting off the entire plant. In instances where the floral clusters mature sequentially, individual harvest is used because the entire plant is not ripe at any given time. Removing individual clusters also makes drying easier and quicker because the stalks are divided into shorter pieces. Floral clusters will dry much more slowly if the plant is dried whole. This means that all of the water in the plant must pass through the stomata on the surface of the leaves and calyxes instead of through cut stem ends. The stomata close soon after harvest and drying is slowed since little water vapor escapes.

Boiling attached Cannabis roots after harvesting whole plants, but before drying, is an interesting technique. Origi nally it was thought by cultivators that boiling the roots would force resins to the floral clusters. In actuality, there are very few resins within the vascular system of the plant and most of the resins have been secreted in the heads of glandular trichomes. Once resins are secreted they are no longer water-soluble and are not part of the vascular system. As a result, neither boiling nor any other process will move resins and cannabinoids around the plant. However, boiling the roots does lengthen the drying time of the whole plant. Boiling the roots shocks the stomata of the leaves and forces them to close immediately; less water vapor is allowed to escape and the floral clusters dry more slowly. If the leaves are left intact when drying, the water evaporates through the leaves instead of through the flowers.

Whole plants, limbs, and floral clusters are usually hung upside down or laid out on screen trays to dry. Many cultivators believe that hanging floral clusters upside-down to dry makes the resins flow by gravity to the limb tips. As with boiling roots, little if any transport of cannabinoids and resins through the vascular system occurs after the plant is harvested. Inverted drying does cause the leaves to hang next to the floral clusters as they dry, and the resins are protected from rubbing off during handling. Floral clusters also appear more attractive and larger if they are hung to dry. When laid out flat to dry, floral clusters usually develop a flattened, slightly pressed profile, and the leaves do not dry around the floral clusters and protect them. Also, the floral clusters are usually turned to prevent spoilage; this requires extra handling. It is easy to bruise the clusters during handling, and upon drying, bruised tissue will turn dark green or brown. Resins are very fragile and fall from the outside of the calyx if shaken. The less handling the floral clusters receive the better they look, taste and smoke. Floral clusters, including large leaves and stems, usually dry to about 25% of their original fresh weight. When dry enough to store without the threat of mold, the central stem of the floral cluster will snap briskly when bent. Usually about 10% water remains in dry, stored Cannabis floral clusters prepared for smoking. If some water content is not maintained, the resins will lose potency and the clusters will disintegrate into a useless powder exposed to decomposition by the atmosphere.

As floral clusters dry, and even after they are sealed and packaged, they continue to cure. Curing removes the unpleasant green taste and allows the

resins and cannabinoids to finish ripening. Drying is merely the removal of water from the floral clusters so they will be dry enough to burn. Curing takes this process one step farther to produce tasty and psychoactive marijuana. If drying occurs too rapidly, the green taste will be sealed into the tissues and may remain there indefinitely. A floral cluster is not dead after harvest any more than an apple is. Certain metabolic activities take place for some time, much like the ripening and eventual spoiling of an apple after it is picked. During this period, cannabinoid acids decarboxylate into the psychoactive cannabinoids and terpenes isomerize to create new polyterpenes with tastes and aromas different from fresh floral clusters. It is suspected that cannabinoid biosynthesis may also continue for a short time after harvest. Taste and aroma also improve as chlorophylls and other pigments begin to break down. When floral clusters are dried slowly they are kept at a humidity very near that of the inside of the stomata. Alternatively, sealing and opening bags or jars or clusters is a procedure that keeps the humidity high within the container and allows the periodic venting of gases given off during curing. It also exposes the clusters to fresh air needed for proper curing.

If the container is airtight and not vented, then rot from anaerobic bacteria and mold is often seen. Paper boxes breathe air but also retain moisture and are often used for curing Cannabis. Dry floral clusters are usually trimmed of outer leaves just prior to smoking. This is called manicuring.

The leaves act as a wrapper to protect the delicate floral clusters. If manicured before drying, a significant increase in the rate of THC breakdown occurs.

Storage

Cannabis floral clusters are best stored in a cool, dark place. Refrigeration will retard the breakdown of cannabinoids, but freezing has adverse effects. Freezing forces moisture to the surface from the inside of the floral tissues and this may harm the resins secreted on the surface. Floral clusters with the shade leaves intact are well protected from abrasion and accidental removal of resins, but manicured floral clusters are best tightly packed so they do not rub together. Glass jars and plastic freezer bags are the most common containers for the storage of floral clusters. Polyethylene plastic sandwich or trash bags are not suited to long-term storage since they breathe air and water vapor. This may cause the floral clusters to dry out excessively and lose potency. Heat-sealed boilable plastic pouches do not breathe and are frequently used for storage. Glass canning jars are also very air-tight, but glass breaks. It is feared by some connoisseurs that plastic may also impart an unpleasant taste to the floral clusters. In either case, additional care is usually taken to protect the floral clus ters from light so another opaque container is used to cover the clear glass or plastic wrapping. Clusters are not sealed permanently until they have finished curing. Curing involves the presence of oxygen, and sealing floral clusters will end the free exchange of oxygen and end curing. However, oxygen also causes

the slow breakdown of THC to CBN, so after the curing process is completed, the container is completely sealed. Any oxygen present in the container will be used up and no more can enter. Nitrogen has been suggested as a packing medium because it is very non-reactive and inexpensive. Jars or bags may be flooded with nitrogen to displace air and then sealed. Vacuum-sealing machines are available for Mason jars and may be modified to vacuum-sealed bags.

The proper harvesting, curing, and storage of Cannabis closes the season and completes' the life cycle. Cannabis is certainly a plant of great economic potential and scientific interest; its rich genetic diversity deserves preservation and its possible beneficial uses deserve more research.

He who sows the ground with care and diligence acquires greater stock of religious merit than he could gain by the repetition of ten thousand prayers.

- Zoroaster, Zendavesta