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STEPS IN BECOMING YOUR OWN PLANT BREEDER

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As herb growers and marketers, all of us enjoy working with plants and their useful products. Many of us are involved with growing herbs from seed or cuttings. There probably aren't quite so many of us who produce our own seed and of those who do produce seed there are even fewer who do so using some method of controlled pollination. To be a plant breeder, first you need to learn how to produce quality seed under controlled pollination conditions for the species you want to improve.

The more you can learn about the breeding biology of your species, the more you will be able to accomplish. So right at the start you need to survey both the scientific literature and seed producers to assemble a body of knowledge on your species' breeding biology. Five important questions that need to be examined while you assemble this information include:

1. What conditions must be met to induce flowering and seed set?
2. Where does your species fall along the continuum between an obligate, self-pollinating type (a plant with flowers that remain closed through pollination, such as *Viola*) to an obligate outcrosser?
3. If your species is normally an outcrosser, are there physiological barriers to self-pollination? (Conversely, if your species is normally self-pollinated, how difficult is it to artificially cross?)
4. How is this species pollinated in nature and is there any way to manipulate the natural pollination system to do your bidding?
5. Do different populations of your species vary in breeding systems?

Later in this talk we will come back to these questions to consider their implications for breeding strategy. Once you know as much as you can about seed production and the breeding system, you need to set some goals.

What characteristics do you want to improve? To have any chance of success you must choose your goals carefully. First you should pick characteristics that are primarily under genetic control. The way a plant looks or tastes is influenced by its genes, by the environment in which it's grown, and by the interaction between the environment and the plant's genes. Breeders can usually make the most progress by working on the improvement of those traits that are not greatly influenced by variation in normal field conditions.

When you begin a breeding project, you need to rely on careful observations or measurements taken on a set of different populations of your species over a number of years or at a number of different locations to get an idea of the balance between genetic control and environmental influences. This approach will also be instructive in that it will expose you to the concept of experimental replication. You can be most sure that differences you see in the field are real and repeatable, if they appear in more than one year and in more than one location. Even within a single test planting, replication (multiple plantings of each line being tested) can be useful, because fields are not always uniform.

After you identify characteristics that are under some degree of genetic control, then you need to find useful genetic variation for those characteristics. A breeder can accomplish nothing without genetic variation. In those cases where useful variation does not turn up, as was the case for *Verticillium* resistance in peppermint (Murray, 1969), the breeder is forced to turn either to other related species that might cross with the one being improved or to using chemicals and/or ionizing radiation to cause mutations. Mutation breeding goes beyond the scope of this talk, but there may be circumstances where you will want to look at it or at other species related to yours. We will return to this when we look at the breeding system questions, since it is difficulty with breeding systems that hampers the use of crosses between related species.

Once you know that the characteristics you're interested in are under genetic control and that there is useful variation for those characteristics, then you can put together your list of goals and develop a breeding strategy. From a breeding strategy perspective, goals generally fall into four categories;

1. Increased uniformity in seed-propagated species;
2. Changing the mean value for a characteristic in a population of a seed-propagated species;
3. Selecting an extreme type for a vegetatively-propagated species;
and
4. New combinations of existing traits.

We can look at these four situations using simple examples and graphs.

1. Let's consider a hypothetical population of 100 dill plants. Dill is a cross-pollinated annual. The grower wants to cut the crop for dillweed just before flowering starts, but flowering time varies from plant to plant and is under a degree of genetic control. In Figure 1, you see that the plants in this population have a statistically normal distribution of first flowering dates. The breeder's goal would be to gradually produce a more uniform population by culling the extremes over repeated generations (Figure 2). The breeder could cull in the field using mass selection or could use a more sophisticated system for culling such as planting separate rows from the seeds of each selected plant and then choosing the plants for the next generation on the basis of the performance of the progenies (also known as half-sib selection).

2. Let's continue with dill. The breeder might want to produce an earlier flowering dill. Still looking at flowering time in the dill population (Figure 3), the breeder, rather than culling extremes, could cull all but the earliest plants. Repeated cycles of this sort of selection can then hasten the mean flowering date (Figure 4). One of the classic examples of this type of selection is the oil and protein selection in corn. After seventy generations of divergent selection for oil and protein concentration, progress was still being made (Dudley et al., 1974).

Note: If your breeding goal is of the first or second sort, and you need to use repeated cycles of selection, one of your main considerations will need to be adequate population size. If you select too small a sample each cycle, two problems can arise. First, over time your plants will become more closely related and inbreeding depression, a loss of plant vigor, may occur and, second, you may limit the progress you can make by not having enough useful variation. If you select too strictly, you reduce useful genetic variation and then you will not be able to make any further gain without bringing in new material.

3. Moving from dill to *Monarda*, let's look at a species that can be vegetatively propagated on a commercial scale using root or stem cuttings. Figure 5 shows a hypothetical curve for variation in percent geraniol in the essential oil of a *Monarda* population. Rather than reduce variation as we did in point 1 or shift the mean value as we did in point 2, here we want to increase the number of plants in the right-hand tail of the distribution. One way we could do this is by selecting a few of the plants with the highest concentration of geraniol and crossing them together. The result often looks like Figure 6. Since we can vegetatively propagate, we can just make selections from the right-hand tail of Figure 6 and propagate them for a replicated trial and make a final choice on the basis of the trial results.
4. In the first three examples, for ease of explanation characteristics were chosen that were under additive genetic control. In other words, many genes with small, cumulative effects combined to control flowering date or geraniol concentration. This type of genetic control is common, but is clearly not the only sort that you will encounter. For the fourth example, let us consider combining characteristics that are under the control of a single gene with a big effect rather than many genes with small, cumulative effects. Generally, traits that are controlled by a single gene can be seen to fall into distinct classes with, at most, a single intermediate class; examples include white vs. pigmented flower color, green vs. red plants, dwarf vs. tall plants, and many insect and disease resistances.

For this final case, let's use an example from corn. If we wanted to produce a red corn plant with gold stripes on the leaves and we had two pure-breeding lines, one with gold stripes and the other with red plants, we could cross the lines together. This hybrid turns out to have the desired combination: a red plant with gold-striped leaves. There is only one problem. If you grow this hybrid in isolation and

harvest seed, the next generation does not breed true. Instead, there are 25% green plants and 25% unstriped plants. To develop a true breeding line, you need to understand how the individual genes controlling these traits work.

Each corn plant has two copies of each gene. It transmits only a single, randomly chosen copy of each gene through its pollen and eggs. The original red plant had two copies of a gene, A, that controls anthocyanin pigmentation and two copies of a gene, og, that produces unstriped leaves. The original striped plant had two copies of a different version of the anthocyanin gene, a, and two copies of the Og gene for gold stripes. The hybrid has both A and a and Og and og. It appears red and striped because A and Og are dominant characteristics. However, you can see that the hybrid has the potential to make green and unstriped plants.

To get a true-breeding line, we need to produce plants that have A A Og Og instead of A a Og og. One way that we can do this is by growing the seedlings produced from an isolated planting of the hybrid. Early on, we can thin out the green or unstriped plants. Later, we self-pollinate the red, striped plants and the next season plant out about 30 seeds from each selfed ear in separate rows. Each row can exhibit one of four patterns. First, some rows will only have red, striped plants. These are from the pure-breeding selfed ears. The other three classes do not have the two traits fixed. Theoretically, one ninth of all selfed ears should produce the desired type, assuming that you culled all of the green or unstriped plants. If we remove the other eight-ninths of the rows, we can then produce isolated seed from the uniform rows. This seed will breed true, producing only A A Og Og.

The corn example is fairly simple because the two characteristics each have only two classes and because they are inherited independently. Breeding for new combinations of characteristics can be much more difficult if either the genes for the two traits are physically linked together on the same chromosome (linkage) or if one gene has an influence on the expression of the other trait (pleiotropy and epistasis).

An introductory text giving an excellent overview of breeding strategies was written by Simmonds (1979). This might be a good starting point for a prospective breeder. More basic introductions to the topic of plant breeding have been published. Two of the better reports are a circular by Butler and Oebker (1966) and a special plant breeding issue of Plants and Gardens (Brooklyn Botanic Gardens, 1974).

Good sources to consult on improving traits that are controlled by many genes (points 1 and 2) include an introductory paper by Schnell (1978) and an in-depth review by Wricke and Weber (1986); for vegetatively propagated crops (point 3) see the compendium edited by Abbott and Atkin (1987) and a review of applications of mutation breeding for these crops (Broertjes and Van Harten, 1978); and for an introduction to single gene models (point 4), linkage, pleiotropy and epistasis check appropriate chapters of the text by Welch (1981).

In addition to your goals, the breeding system of your plant can have a major influence on your choice of breeding strategies.

What resources will it take to produce quality seed from controlled crosses? As you consider your breeding goals, you need to be realistic in evaluating the time, labor, and growing conditions necessary to achieve those goals. This is where the breeding system questions really become important.

Returning to the original five questions, first you need to fine tune the flowering period of your plants so that you are able to produce quality seed. You may find that your growing season is too short to produce seed from certain varieties. In such a situation, a greenhouse could be a useful addition to your program.

If you want to cross different populations together, you may need to manipulate the populations so that their flowering periods overlap. This can sometimes be done for annuals by using repeated, staggered planting dates. Biennials may require an artificial vernalization to synchronize flowering and to avoid overwintering losses. With perennials, you may want to investigate the effects of artificial daylength manipulation or plantings at more than one site to get the overlap you need (Major, 1980). The other alternative for crossing plants with different flowering periods is a crash course on pollen storage techniques (Stanley and Linskens, 1974).

The other four questions deal more directly with breeding systems. Knowing the natural breeding system of your species can be especially helpful when you need to make controlled pollinations. There are three kinds of controlled pollinations: two clear cases, controlled self-pollination and cross-pollination with a known pair of parents, and a mixed system where an open-pollinated population is increased in isolation. We have seen situations where the breeder uses each of these types of crosses in the examples I gave with the four kinds of breeding goals.

For a predominantly selfing species, such as *Perilla*, selfed seed is easy to produce. You may want to experiment with inflorescence bagging or isolated, single plants to get pure selfed seed. A controlled outcross is a different matter. First you must stop the natural process that leads to self pollination. Physical emasculation, the removal of a flower's anthers before they can shed pollen, is a "low-tech" approach. Then you need to collect pollen from your male parent and transfer this pollen to the emasculated flowers. This transfer must be done in such a way that the pollen can germinate and grow down the style to fertilize the female parent. Hand pollination of self-pollinated species is labor intensive and breeders are always searching for male sterility systems to replace hand emasculation and for more efficient pollen transfer methods. Open-pollinated varieties of self-pollinated species are generally uniform, unless they are intentional or accidental mixtures and selection within these populations is usually not effective, except to cull off-types.

The cross-pollinated species present different problems. Controlled selfing may be quite difficult. In the plant kingdom, there are number of different mechanisms to enforce outcrossing including dioecious plants (plants with only one sex), plants that have pollen that is shed at a

different time than when the stigmas are receptive, and physiological self-incompatibility. Each of these barriers is sometimes circumvented. There are chemical treatments to overcome dioecy in Cucumis (Frankel and Galun, 1977). Pollen storage can be used to avoid timing problems (Stanley and Linskens, 1974). And breeders have used techniques such as heat treatments and cut styles to break down physiological self-incompatibility barriers (Ascher, 1976).

Some plants are primarily cross pollinated, but do self fairly easily. For these plants, the main problem is controlled crossing. You need to use the same emasculation precautions that you would for self-pollinated species. For most outcrossers, isolated open-pollinated increases are generally not a problem, assuming that appropriate pollen vectors are present.

A notable review surveying the relationship between reproductive biology and plant breeding has been compiled by Frankel and Galun (1977).

If you find that you do not have the variation present to achieve your goals within your target species, you may look to related species and attempt a breeding program based on moving useful characteristics from a related species to your target species. This can lead to many breeding system and seed production problems. Interspecific hybrids are usually considerably less fertile than their parents. Repeated cycles of selection for fertility, the doubling of chromosomes using colchicine, and embryo rescue techniques are just a few of the methods that plant breeders employ to overcome fertility problems. A good general reference work summarizing research on this topic was written by Hadley and Openshaw (1980).

As you can now see, there are many challenging steps along the way to becoming your own plant breeder. In this endeavor, there are two clear tasks: to define your breeding goals and to learn as much as you can about the breeding biology and genetics of your plant. Once you have armed yourself with well-defined goals and an understanding of the plant, then a bigger challenge awaits you: designing and, over time, refining an appropriate breeding program to achieve your goals.

If this seems a bit overwhelming, just remember that the initial domestication and early improvement of our major crops were made without the benefit of modern biological tools. And many of our most attractive ornamentals, such as roses and rhododendrons, are currently bred by amateurs. Skillfully designed breeding projects for herbs could produce even more impressive results. The field is wide open.

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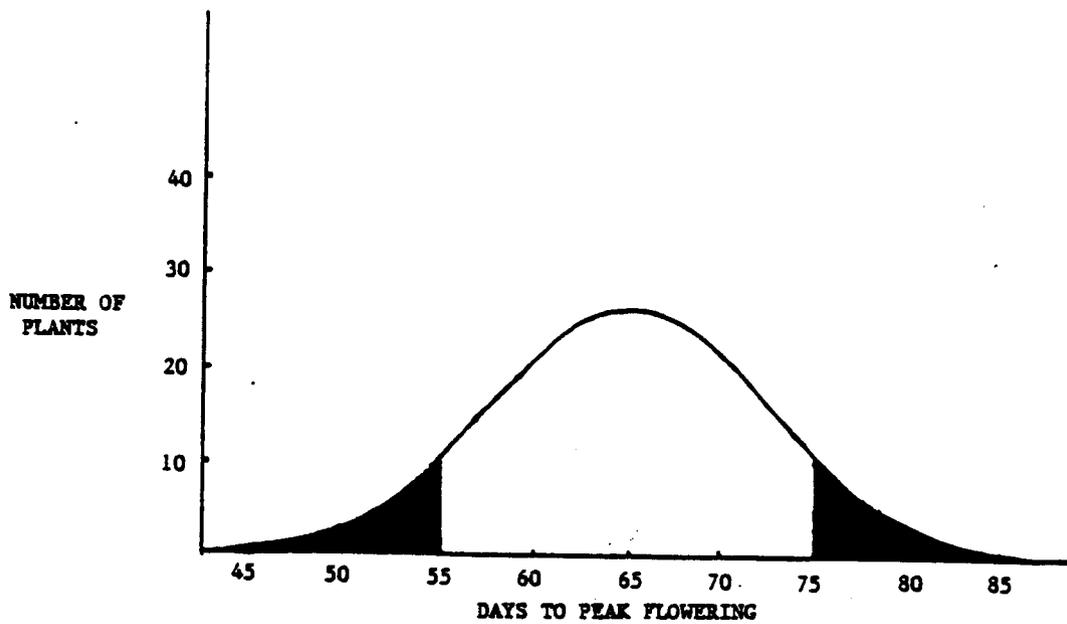


Figure 1. Relationship between number of plants and peak flowering date in a hypothetical dill population. Shaded area indicates the plants culled in selection.

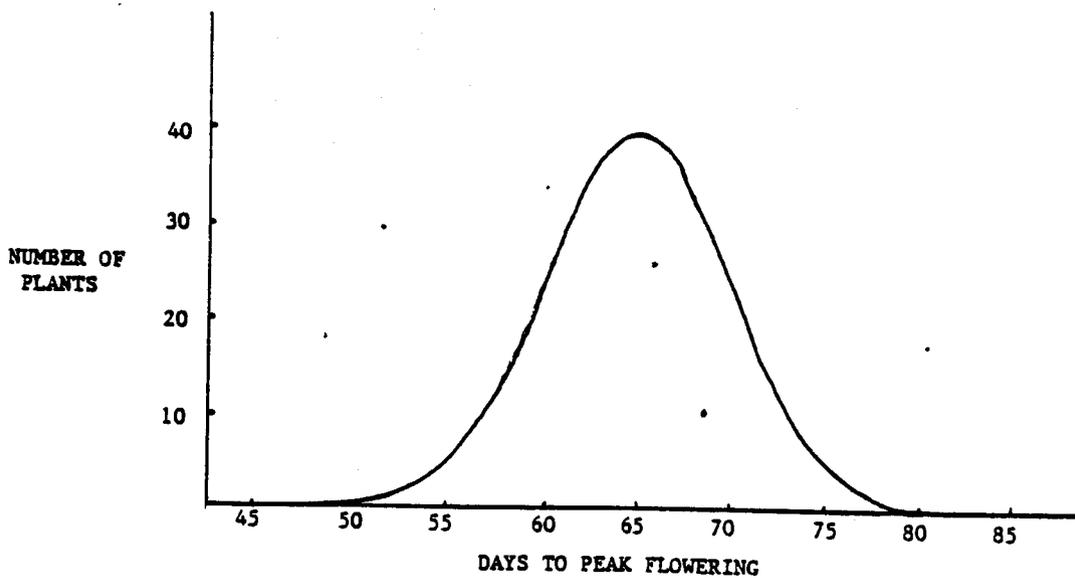


Figure 2. Relationship between number of plants and peak flowering date in a selected dill population. (Point 1 - selection for more uniform flowering)

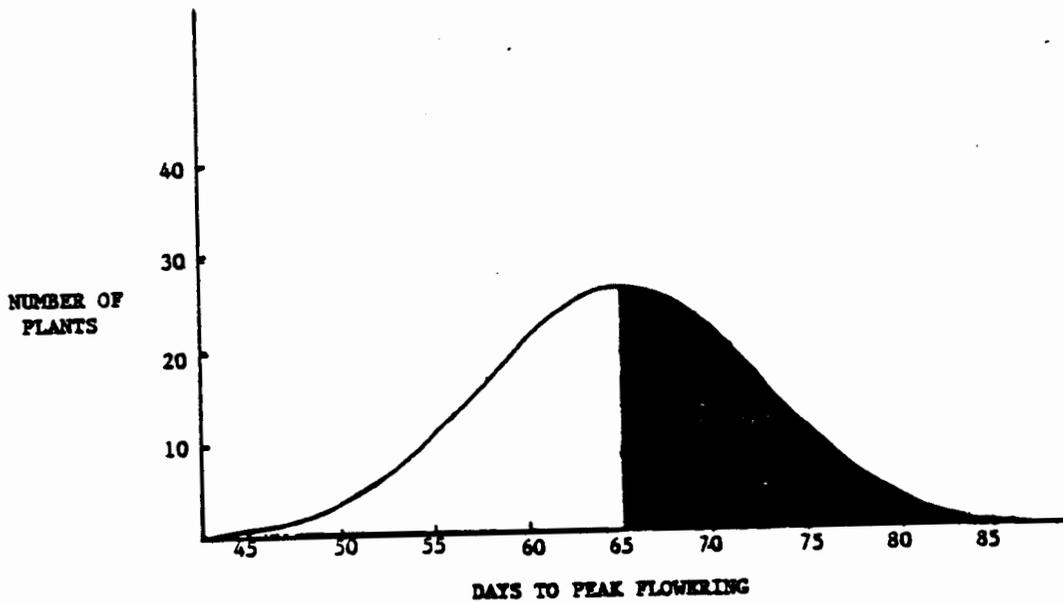


Figure 3. Relationship between number of plants and peak flowering date in a hypothetical dill population. Shaded area indicates the plants culled in selection.

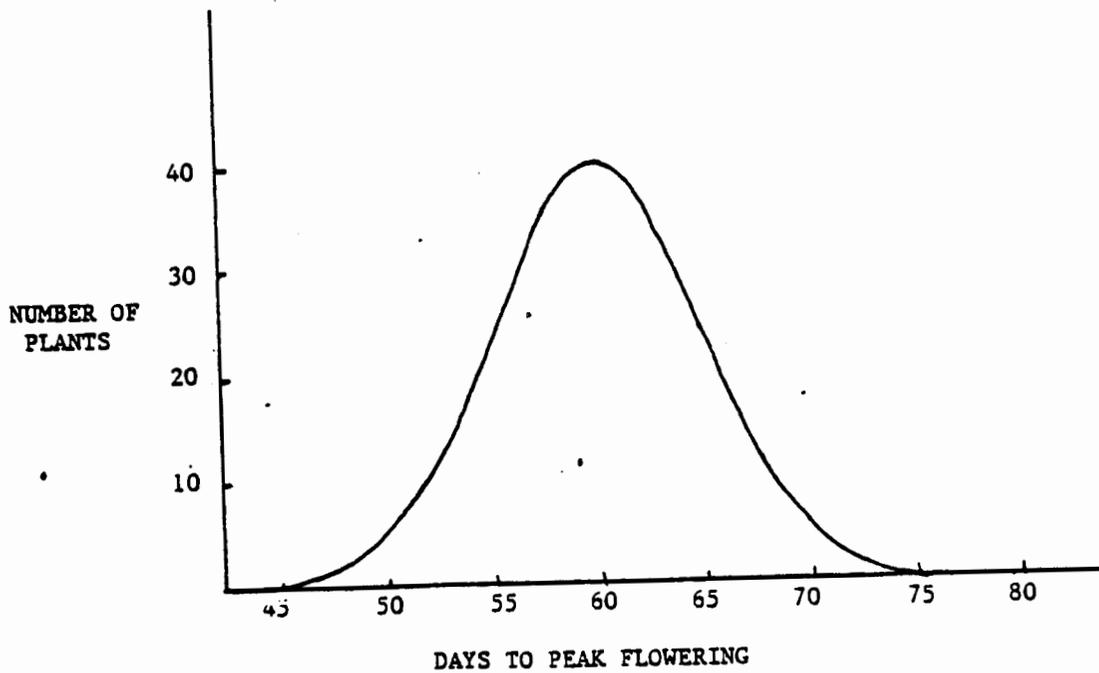


Figure 4. Relationship between number of plants and peak flowering date in a selected dill population. (Point 2 - selection for earlier flowering)

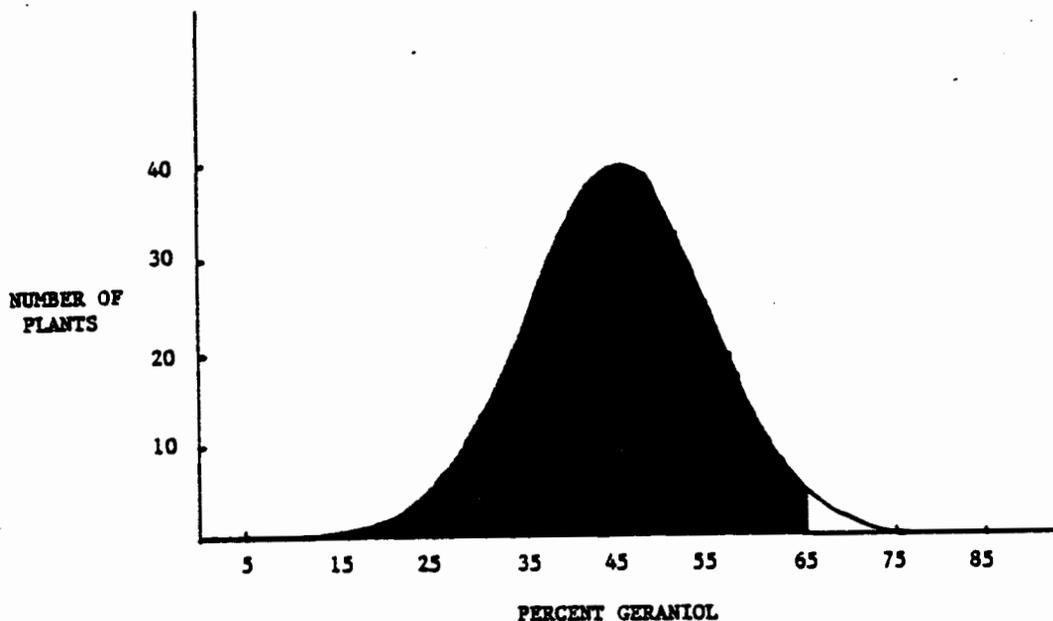


Figure 5. Relationship between number of plants and geraniol concentration in a hypothetical Monarda population. Shaded area indicates the plants culled in selection.

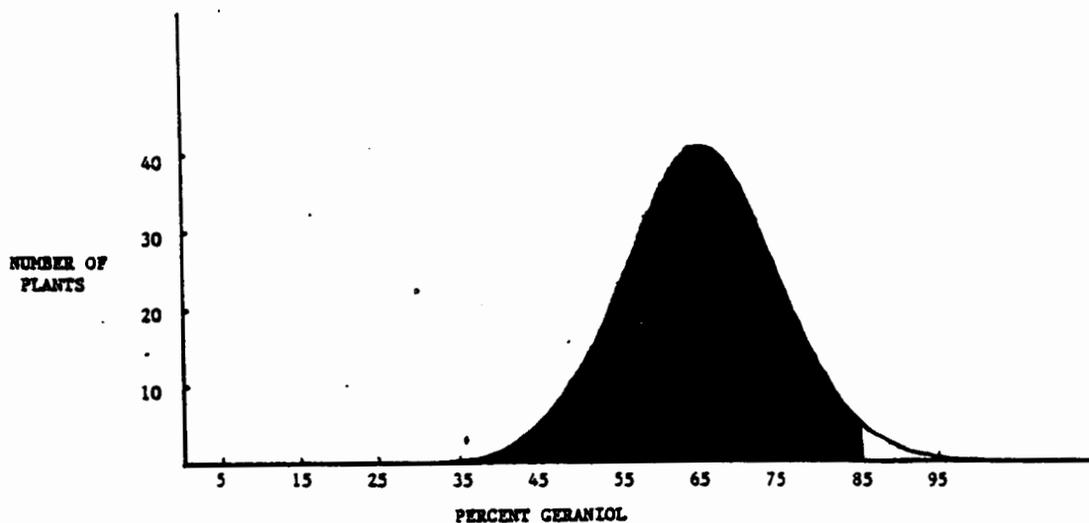


Figure 6. Relationship between number of plants and geraniol concentration in a selected Monarda population. Unshaded area indicates plants to be vegetatively propagated for a replicated test. (Point 3 - selection for extreme type followed by vegetative propagation)