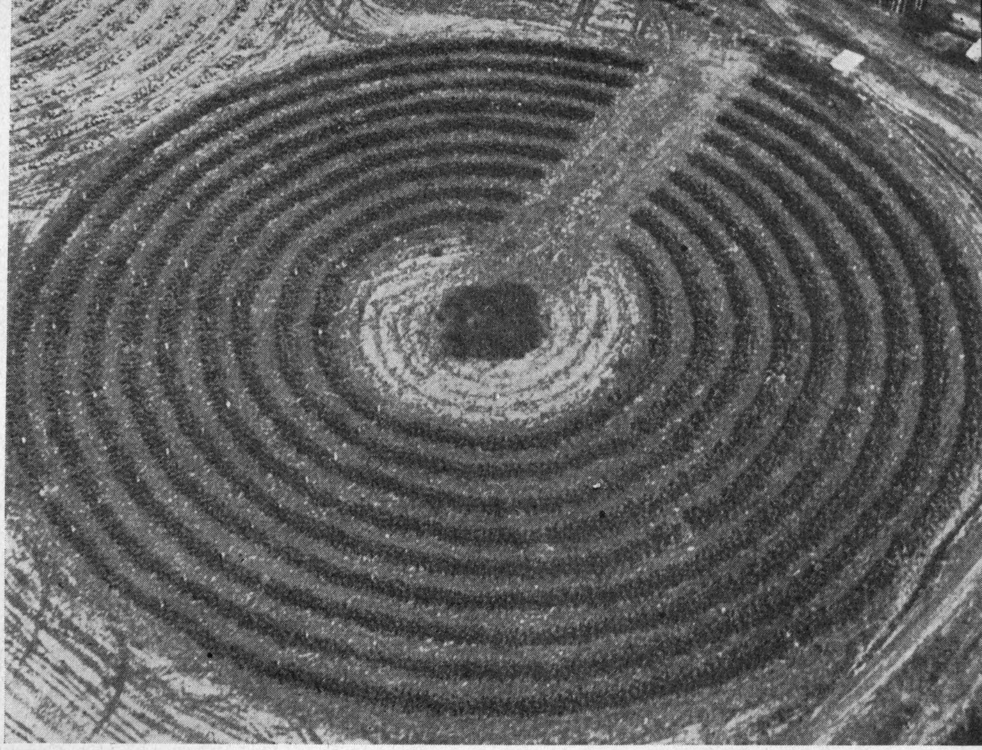
BULLETIN 530



APRIL, 1949

SPIRALLY ARRANGED PLOTS IN A DESIGN FOR FIELD ASSAY OF FUNGICIDES

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Cover ***Illustration.*** **View of a spiral of celery. Photograph taken from a helicopter.**

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Spirally Arranged Plots in a Design for Field Assay of Fungicides

James G. Horsfall and Saul Rich[1]

Drudgery makes life hard for the investigator of the protective action of fungicides in the field. This paper, a companion piece to those already published on laboratory assay of fungicides (1-6, 8), will describe a technique which has eliminated for us some of this toil.

The primary source of drudgery in the field assay of fungicides is distance. If many treatments could be tested on a single plant, distance would not be a factor. Many treatments mean many plants, even if only one plant per treatment is necessary. The more plants there are, the farther apart they must be, the more heterogeneous is the soil, the more variable is the inoculation, and the farther the spray and spray machinery must be toted.

The more heterogeneous the plants are, the more plants there must be to compensate for the differences. When more plants are used, the plots must be spaced still farther apart and the chance for differences becomes greater. The more plots the operator must spray, the farther he must be from his supplies, the longer the distances to be travelled and the greater is the drudgery.

In our attempts to reduce drudgery, we tried various expedients before arriving at the technique to be described. Sifting through our trials and errors, some of the principles of the design now become evident.

To overcome the distance effect, the experimental area was limited, so that everything else had to be scaled accordingly. A spraying device was required that was adapted to small amounts of materials, that was auto-powered and light in weight, and that was capable of easy and rapid change of materials. The size of the plots and the mean distance between them had to be kept to a minimum. We found that the plots in this design could be as small as 10 feet long and one row wide.

By the laws of geometry, the mean distance between plots in any given area is the least within a circle. Therefore, if the field plots are arranged within a circle, the differences between plots and the amount of travel will be reduced to the minimum amount consistent with the number of treatments to be used. Not only are soil differences reduced, but the distance that the fungus must spread after its establishment is also reduced to the minimum. This last point is particularly important in view of the fact that disease decreases logarithmically with distance from the primary source of inoculum as Zentmyer, *et al.* (11) have shown.

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[1] Plant Pathology Department.

SUITABILITY OF SPIRALLY ARRANGED PLOTS

The arrangement of plots along an Archimedean spiral within the circle (cover) has proved to be a practical method of planting and cultivating.

The spiral arrangement has other interesting possibilities to reduce distance as listed below:

1. The spray machine can be spotted at the center of the spiral. At that point it is the closest to the average plot that it can ever be.

2. The spray machine can be stationary. This saves the operator's muscles.

3. A rotating boom may be used to support the spray hose. This also saves energy and delivers spray equally to all plots within the circular area.

4. The travel between the last replicate of one treatment and the first replicate of the next treatment is negligible because the first and last replicates, being on a circle, are contiguous.

5. Irrigation is accomplished by the use of a rotating overhead sprinkler placed at the center. With plants in a spiral, there are no dry corners.

6. The overhead irrigation helps spread the test organism and keeps the plants wet following inoculation.

7. The spiral arrangement helps to minimize the effect of spray drift because there are a minimum number of plots crossing the direction of the prevailing wind.

8. Planting and cultivation is simplified in a manner to be described.

9. The spiral arrangement of small plots yields data having a high order of precision.

PLANTING THE SPIRALS

Each spiral is 80 feet in diameter. Each has at its center riser pipes for water supply and for drainage. The drainage riser serves as the locus for the establishment of the spiral each year. The planter or marker is tied to a cable that winds around a fixed drum which is anchored to the drainage riser at the center. Planting begins in a clockwise direction at the periphery of the spiral and as the planter goes around the center, the cable winds on the drum and pulls the planter inward at a uniform rate. The drum used at the Mt. Carmel Experimental Farm is 11.1 inches in diameter. This establishes a three-foot interval between laterally adjacent plots. Nine complete revolutions are made around the pivot leaving a central working area 26 feet in diameter. This planting procedure produces a continuous spiral row which can be broken into single row plots. It has been possible to lay out and plant five spirals of beans with a hand-pushed planter in a single afternoon.

A 10-foot roadway is left from the outer edge to the center of the spiral for moving in the trailer-mounted sprayer. After subtracting the space for the roadway, each spiral contains 1,320 feet of plants or exactly one-fourth mile. This turns out to be a convenient number. By making each plot 10 feet long, there are 132 plots. By using four replicates, there are 33 plots each. Taking out one for a non-treated check, 32 are left. Thirty-two is still a very useful number because it is divisible by 32, 16, 8, 4, 2 and 1. From that, one can use numerous combinations: 32 treatments, one dose each; 16 treatments, two doses each; 8 treatments, four doses each, or any combination thereof.

The plots are randomized in each quadrant, making four replicated wedge-shaped blocks. The arrangement is such that the first plots in each row are in a straight line at the beginning of the first and third quadrants, but form a "sawtooth" design at the beginning of the second and fourth quadrants.

CULTIVATING THE SPIRALS

The spirals may be cultivated by a wheel hoe or a close-coupled garden tractor. Mulching between the rows reduces soil compaction and weed growth, thus keeping cultivation to a minimum.

WATER SUPPLY

When the spirals were first tried, water from the nearest hydrant was delivered to the spiral with garden hose. The spirals now have permanently installed underground water pipes of ½-inch galvanized or black iron, placed at least 2½ to 3 feet beneath the ground surface so as to avoid the plowshare during plowing. These pipes are laid so that they will drain away from the spirals and, at convenient locations, there are plugs for drainage of the pipes during the winter months.

SEWAGE SYSTEM

A simple sewage system which has been used successfully is a dry well or sump into which the excess spray material is drained directly from the spray tank. The used dry well may be cleaned out at the end of the season and refilled with clean soil in order to avoid poisoning the land. Permanent drains of 1¼-inch pipe have now been installed below the plow sole. These drain pipes run from the center of each spiral downgrade to the sump, then into a tile line and from there into a permanent dry well which is located under one of the farm roads. This location for the permanent well prevents the poisoning of the ground under cultivation.

IRRIGATION

A valve and plug arrangement in the sump (Figure 1) allows the same pipe to be used either for drainage or for irrigation. The drain pipe is connected to the water system through the valve. The drainage plug is placed on the spiral side of the valve. For drainage, the valve is closed and the plug taken out so that the waste chemicals can drain from the spiral into the sump and then into the tile. To irrigate, the plug is placed in position and the valve opened so that the water runs up through the pipe to the center of the spiral. For irrigation, the sprayer is removed and a revolving type overhead irrigator is placed on the drain riser. Water is applied at night when there is less wind and, hence, less variability in the overhead irrigation. The irrigation is controlled by means of a time-clock on a motorized valve.

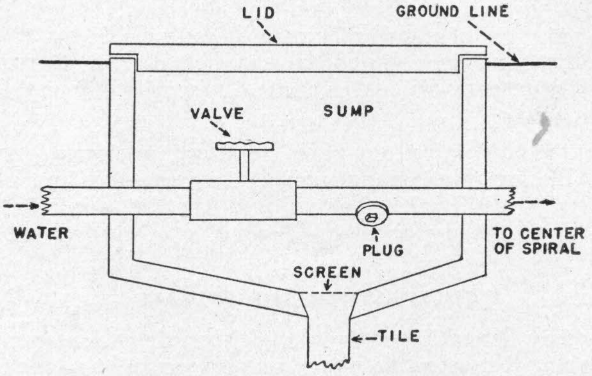

Figure 1. Diagram of valve and plug arrangement at the sump. Valve is open and plug is in when the line is used for irrigation. Valve is closed and plug is out when the line is used for drainage.

SPRAYING EQUIPMENT AND TECHNIQUE

The sprayer is a restyled John Bean, cub model, equipped with a pump with a capacity of six gallons per minute, and mounted on a rubber tired trailer so that it may be moved from spiral to spiral.

It is provided with two 15-gallon tanks, making it possible to mix in one tank while spraying from the other. Each tank has an external glass gauge calibrated in gallons. This allows a direct reading of the volume of

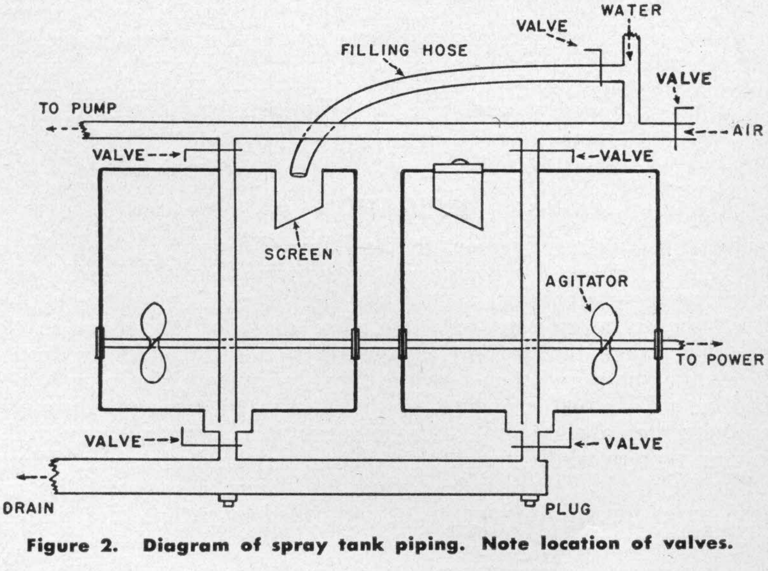

Figure 2. Diagram of spray tank piping. Note location of valves.

liquid within the tanks, which is useful in making dilutions. The sprayer must be level if these gauges are to be accurate. Therefore, the sprayer is equipped with two leveling bubbles at a right angle for horizontal leveling, both laterally and longitudinally.

The drainage runs down from each tank through valves into a single pipe attached by means of a 1¼-inch rubber hose to the drainage riser in the center of the spiral. Likewise, the suction lines from each tank connect through valves to the pump (Figure 2). The suction lines also connect through valves to the incoming water line for flushing. Another valve admits air to the pump for clearing out spray material and flush water. Finally, a short length of ½-inch garden hose to the water line permits the operator to wash the spray materials through a screen into the tank. All valves are the so-called lever-action, quick-acting type.

The spray must be delivered to all parts of the spiral, including the periphery, 40 feet from the center. This is accomplished by supporting the spray hose in an overhead cantilever aluminum pipe which is free to rotate (Figure 3).

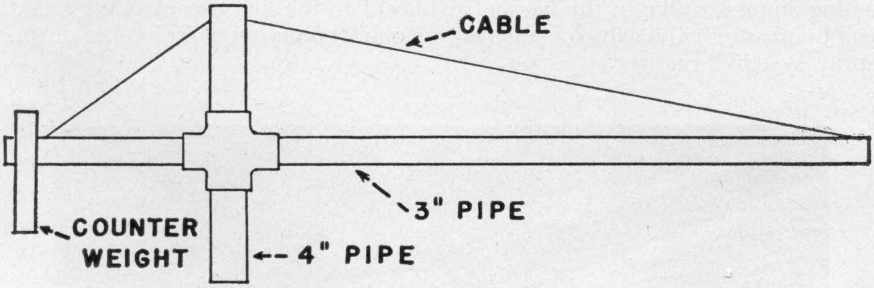

Figure 3. Diagram of boom used to hold spray hose above the plots.

The long arm of the boom is 20 feet. This is about the longest practicable dimension. The spray hose passes through the inside of the aluminum pipe. Where the hose enters the bottom of the upright support, it is provided with a swivel connection so that the boom can rotate freely. The hose extends about 22 feet beyond the end of the boom so that the operator can reach the outermost plant 40 feet from the center. A second swivel connection located at the point where the hose leaves the outer end of the boom prevents the hose within the boom from twisting as the boom rotates.

The boom is mounted as an integral part of the sprayer so that it can be transported with the sprayer. It is mounted six feet above the ground to allow head clearance for the operators.

Any hand-held spraying device may be used with the spiral equipment. At Mt. Carmel, various types of sprayers are used. One of these is a three-nozzle sprayer with one nozzle directed vertically downward, and the other two nozzles directed horizontally inward (Figure 4). This device is used when the material being applied is already in a wettable form and readily available. The three-nozzle sprayer is controlled by a quick-

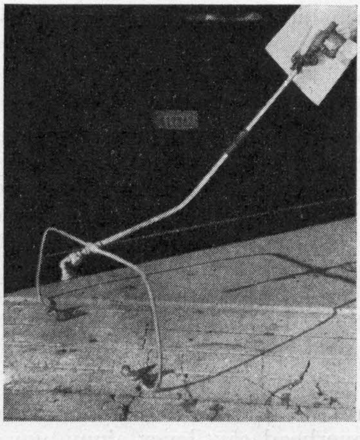

Figure 4. Three-nozzle spraying device. Top nozzle sprays downward. Side nozzles spray inward.

acting squeeze valve in the handle. Adjacent to the liquid-controlling valve is a by-passing valve which is opened for quick emptying and cleaning of the spray system (Figure 5).

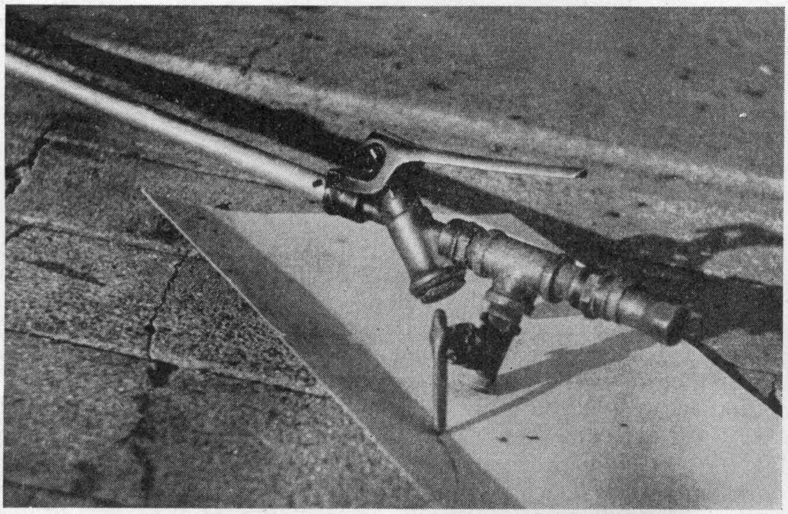

Figure 5. Close-up of valves on handle of three-nozzle sprayer. Squeeze valve at top is for controlling flow of spray to nozzles. Valve pointing downward is opened to release pressure during cleaning of the pump and line system.

Drift, the bugaboo of so many field experiments, has been found to be negligible in our trials. Spring-loaded check valves (Figure 6) are placed immediately behind each of the three nozzles. When the spraying is being

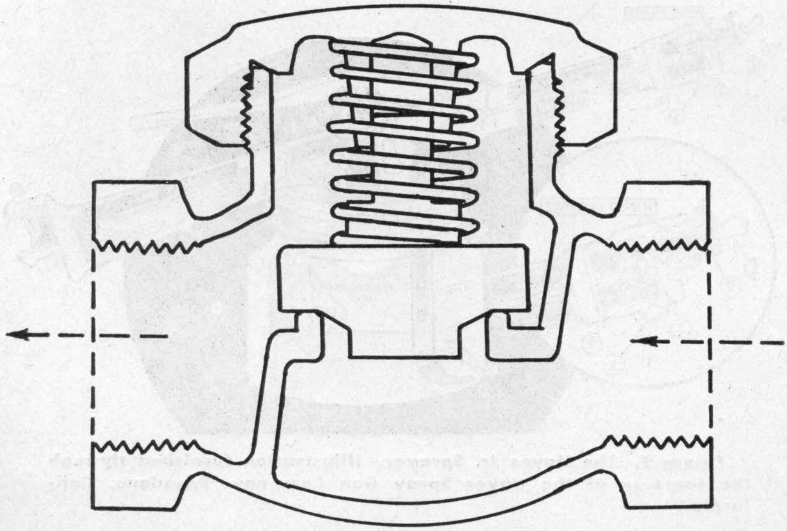

Figure 6. Drawing of a spring-loaded check valve. (Diagrammatic sketch from an illustration furnished through the courtesy of Jenkins Brothers, Manufacturers of Valves, Bridgeport, Connecticut.)

done, the pressure of the spray liquid compresses the springs, forcing open the valves and allowing the liquid to pass through the nozzles. When the spray material is shut off, the pressure of the springs forces the check valves closed and thus prevents any drizzle from the nozzles as the operators move from plot to plot.

The lessening of the drudgery has allowed for much more accurate spraying throughout the whole spray period so that the treatment effects cut off sharply at the plot terminals. Season after season, good treatments next to ineffective treatments stand out clearly, with no apparent drift effect in any direction. The lack of drift error may be explained by: (1) accuracy in spraying; (2) the use of the spring-loaded check valves to prevent drizzle, and (3) the fact that the smaller sized droplets, which tend to drift the most, do not stick to foliage, but actually bounce (10).

The Hayes Jr. sprayer, another spraying device used in the spirals, helps solve two of the serious problems in field testing of new organic compounds: (1) poor wettability and (2) insufficient quantity of sample. Usually an attempt is made to answer the first problem by formulating the compounds with wetting and suspending agents. In such cases the researcher cannot distinguish the inherent performance of the material from the performance of the formulation. The usual solution for the second problem is to wheedle additional material from the chemists.

The Hayes Jr. sprayer (Figure 7) is a liquid proportioner designed primarily for home gardeners, and intended for use with garden hose. This sprayer has a small reservoir from which the spray concentrate is injected into the water stream near the nozzle by means of a Venturi jet. The non-wettable materials can be dissolved in acetone, isopropyl alcohol,

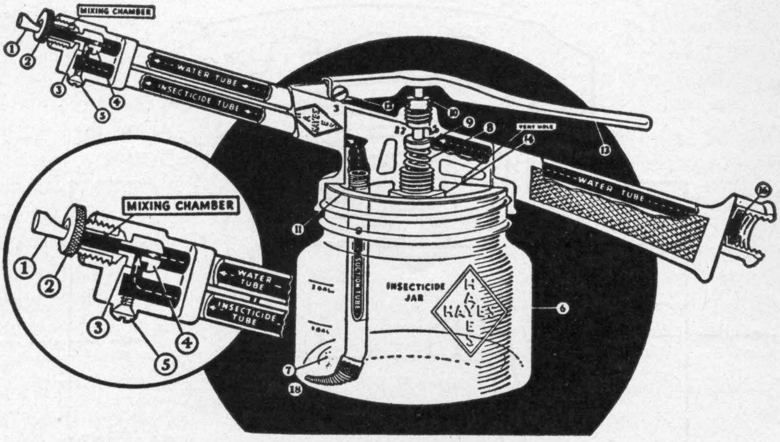

Figure 7. The Hayes Jr. Sprayer. (Illustration furnished through the courtesy of the Hayes Spray Gun Company, Pasadena, California.)

or some other suitable organic solvent, and are conveniently suspended in water by the sprayer at the Venturi jet immediately prior to deposition on the plant. Spraying check plots with solvents alone has demonstrated no adverse effects from these materials. The Hayes sprayer should be carefully cleaned and calibrated before each use and cannot be used with gummy materials which would clog the small Venturi hole.

The use of the Hayes sprayer, or any other liquid proportioner, makes possible the application of materials without complex spray rigs. As pres-

Figure 8. Spraying the spiral plots.

sure is relatively unimportant in the application of spray materials to such crops as bean or celery, the Hayes sprayer can be attached directly to the water source and water line pressure may then be used for spraying. It is important that the sprayer be calibrated carefully with the materials and pressures to be used, because pick-up by the Venturi jet varies with the water pressure and with differing viscosities of concentrates.

The actual spraying operations require three men when the three-nozzle device is used (Figure 8) and two men when the Hayes sprayer is in use. For the three-nozzle outfit, one man handles the mixing and draining of the spray materials at the spray tanks in the center of the spiral, one man carries the extra hose and directs the spray man to the proper plots by means of a spray-plot diagram, and one man does the actual spraying. When the Hayes sprayer is in use, no mixing is required at the central spray tanks so that only the spray man and hose man are required.

In the spraying operation using the three-nozzle sprayer, air is passed through the pump and spray line when changing from one concentration to another of the same material. In dosage series, it is advisable to go progressively from the lowest concentration to the highest concentration. This greatly reduces the error arising from possible contamination. Between changes of material, the air is followed by cleansing water to lessen the chances of mixtures of materials in the spray system.

Although ordinarily the spraying of only two spirals is planned for each day, three spirals can be sprayed in an eight-hour day when necessary. The spraying of three spirals, or 396 plots, means 94 treatments replicated four times. Previous to the advent of the spiral design at Mt. Carmel, only 20 treatments replicated four times could be applied in a day, using knapsack-type sprayers on rectangular plots, with two or three men working. Recent fungicide trials on rectangular plots at Mt. Carmel, using two operators on tractor-drawn equipment, allowed the spraying of only 240 plots in an eight-hour day, or 60 treatments replicated four times.

CROPS AND DISEASES USED

The crop used in the spirals must be low enough so that the operators can move freely over the plants from row to row, or else the individual plants must have enough space between them, e.g., as in planting roses, so as not to impede the movements of the sprayers. Preferably the plants should not be so large as to run together between the rows. Snapbeans, celery, tomatoes, potatoes and roses have been used successfully in the spiral design. The test diseases are all of the foliage-infecting types, such as bean anthracnose, celery *Cercospora* and *Septoria,* tomato defoliation, potato early blight and late blight, and rose black-spot. It is possible to raise two crops of beans in the same spiral and thus obtain two sets of data in a single season. Potatoes, tomatoes and roses are arranged five plants per 10-foot plot, while celery and beans are planted 20 plants per 10-foot plot.

Low wire frames may be used to confine and support otherwise straggling plants such as potatoes or tomatoes. Potatoes have been used successfully in this design when four-foot aisles were left between laterally adjacent plots.

DATA TAKING

Data taking is usually done by the grading system of Horsfall and Barratt (7). Accurate results have been obtained with this technique. Readings are not begun until the disease is general throughout the spiral. The small sized plots allow for rapid spread of disease which might start as a localized infection. The probability of a disease spreading only 80 feet is much greater than is the general infestation of large conventional plots. The chances for heterogeneity are less in the smaller plots, although it does occur. Border effects are likewise diminished.

Various methods of taking data have been tried for individual crops. Anthracnose on beans may be scored on the basis of individual pods removed from the plant, or on the basis of plant unit estimations. In the former technique, 100 pods are read from each plot. In the latter technique, a single reading is made for each of the 10 center plants, or 10 readings per plot. The pod unit method is tedious, and the high degree of accuracy obtained is probably not necessary for most experiments. The plant unit method is much faster, allowing a spiral of beans to be read in one-half day, compared to four or five days for the pod unit method.

Celery plants may be treated as individual stalks or as individual plants. In the stalk method, the plants are necessarily destroyed to get at the individual stalks, while in the plant method the celery remains *in situ* and many readings may be made through the season in order to follow the progress of the disease, and to measure fungicidal efficacy under increasing inoculum potential. Potatoes, tomatoes and roses are read as plant units.

For any of the crops described, a spiral (132 plots) may be read in one-half day using the plant unit method. The spirals are read either by rows or replicates, depending on the amount of time available. If there is sufficient time to finish the spiral, the row reading technique is employed, starting at the outside row. If there is not sufficient time to read an entire spiral, the readings are taken so that whole replicates are read on the same day. Usually, readings are made so that the reader does not see the plot number until he has finished taking data for that plot, thus removing all chance for unconscious bias.

PRECISION OF DATA

As experimental plots may be sprayed with either the Hayes sprayer or the three-nozzle sprayer and data may be taken on the basis of either the plant unit or the plant part unit (bean pod, celery stalk, etc.), it is of interest to present analyses of variance for various representative sets of data. Analysis 1 is based on the discriminant function of three separate readings made on celery infected with *Cercospora,* sprayed with the three-nozzle device and data read on the plant unit basis. Analysis 2 is of data from a bean spiral sprayed with a Hayes sprayer and based on pod unit readings. Analysis 3 is based on a bean spiral sprayed the same as that used for Analysis 2 but the readings were made on a plant unit basis. Analysis 4 is based on data from a bean spiral infected with anthracnose, read on the plant unit basis and comparing the Hayes sprayer with the three-nozzle sprayer. In each case, three separate fungicides were applied at four doses.

All analyses show treatment effects significant above the 99:1 level. In two of them, Analyses 2 and 4, the treatment effects are highly significant in spite of the significant block effects. Analysis 4 shows that the Hayes sprayer and three-nozzle sprayer cannot be used interchangeably but that comparisons can be made only between plots sprayed with the same device.

ANALYSES OF VARIANCE

ANALYSIS 1[1]

Discriminant function for celery *Cercospora* data. Plots sprayed with three-nozzle sprayer and readings taken on plant unit basis. (Spiral H, 1947)

Source	Df	SS	MS	F
Between blocks	3	648.33	216.11	1.18
Between treatments	32	164,427.33	5,138.35	28.00[2]
Blocks x treatments	96	17,613.67	183.48	
Total	131	182,689.33	1,394.58	
Correction	1	806,523.67		

ANALYSIS 2

Data for bean anthracnose. Plots sprayed with Hayes sprayer. Readings taken on pod unit basis. (Spiral B, 1947)

Source	Df	SS	MS	F
Between blocks	3	1,076.32	358.77	10.40[2]
Between treatments	32	21,083.48	658.86	19.10[2]
Blocks x treatments	96	3,312.25	34.50	
Total	131	25,472.05		
Correction	1	223,790.24		

ANALYSIS 3

Data for bean anthracnose. Plots sprayed with Hayes sprayer. Readings taken on plant unit basis. (Spiral D, 1948)

Source	Df	SS	MS	F
Between blocks	3	0.68	0.23	0.73
Between treatments	32	81.23	2.54	8.20[2]
Blocks x treatments	96	29.70	0.31	
Total	131	111.61		
Correction	1	990.02		

ANALYSIS 4

Data for bean anthracnose. Hayes sprayer compared to three-nozzle sprayer. Readings taken on plant unit basis. (Spiral E, 1948)

Source	Df	SS	MS	F
Between blocks	3	7.77	2.59	6.31[2]
Between treatments	23	72.12	3.14	7.66[2]
Method of application	1	3.84	3.84	9.37[2]
Others	22	68.28	3.10	7.56[2]
Blocks x treatments	69	28.28	0.41	
Total	95	108.17		
Correction	1	601.00		

[1] Analysis 1 was prepared under the supervision of Dr. C. I. Bliss.
[2] Significance at 1 per cent level.

In our work on dosage-response curves we have been hampered by scatter about the line. When scatter is large, differences between treatments, and especially differences between slopes of curves, are hard to demonstrate.

The following experiment (9) is typical of the information which may be obtained through the use of the spiral plots. Table 1 presents data from a *Cercospora*-infested celery spiral wherein Dithane D-14 (plus zinc sulfate and lime) was applied at four dosages, at each of three different spray intervals. The data, when graphed on log-probit paper (Figure 9), give excellent straight line responses showing a minimum of scatter.

TABLE 1. DISEASE READINGS OF *Cercospora*-INFECTED CELERY SPIRAL SPRAYED IN A DOSAGE SERIES AT THREE DIFFERENT TIME INTERVALS. DATA GIVEN AS PER CENT HEALTHY.

Material	Conc./100 gal.[1]	3½ days	7 days	14 days
D-14 + $ZnSO_4$ + lime	½ qt.-¼-⅛	94.0	44.0	38.5
D-14 + $ZnSO_4$ + lime	1 qt.-½-¼	96.0	63.5	44.0
D-14 + $ZnSO_4$ + lime	2 qts.1-½	97.5	85.0	51.0
D-14 + $ZnSO_4$ + lime	4 qts.-2-1	98.1	95.0	70.5

[1] $ZnSO_4$ and lime expressed as pounds.

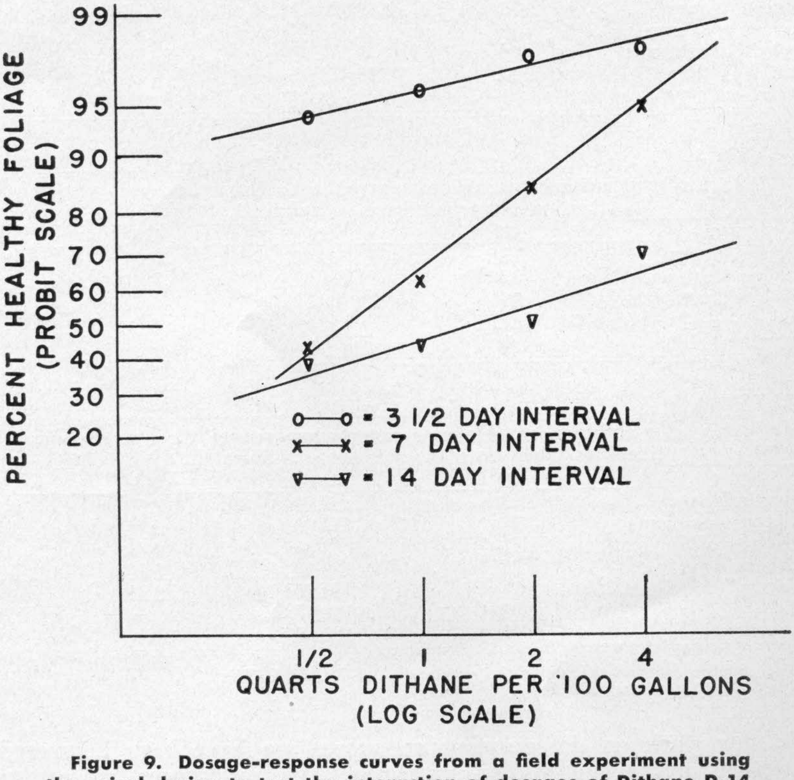

Figure 9. Dosage-response curves from a field experiment using the spiral design to test the interaction of dosages of Dithane D-14 vs. spray intervals. The test organism was *Cercospora* on celery. Note goodness of fit.

The data for the seven-day interval show an unexpected change in slope of the dosage-response curve. This would not have been detected with a less precise technique.

DISCUSSION

The use of the spiral design and its associated techniques have taken much of the drudgery from the field testing of pesticides. This relief from tedium has made possible increased accuracy in the use of small plots, especially in circumstances wherein the actual spraying is performed by supervised, but disinterested, summer help. The more difficult and physically tiring the routine task, the less accurate is the performance of that task as the working day progresses. The increased mental acuity of the operators through the lessening of physical labor, then, is considered one of the foremost advantages of the spiral design. The lessening of heterogeneity by diminishing of the distance factor is the other main advantage.

The speed of application gives the operators the time required every day for the weighing of the spray materials, and other odd jobs arising during the operation.

It must be reiterated that *permanent* installations are not necessary for the establishment of spiral plots. Not only can the water supply be carried in garden hose, and the excess spray material drained into dry wells, but also the boom that supports the hose may be made of wood or some other material less expensive than aluminum.

The spiral arrangement lends itself to great flexibility of experimental design. It has been used for many types of experiments other than fungicide screening. Fundamental pesticide investigations employing this design have been made on the interrelated effects of tenacity, coverage, spray interval, pump pressure, nozzle size, and many other factors. Insecticides, as well as fungicides, have been successfully tested in spiral plots.

SUMMARY

1. A spiral arrangement of experimental plots is described.

2. The spirals are planted by tying the planter or marker to a cable that winds around a fixed drum which is anchored at the spiral center.

3. The spiral arrangement at the Mt. Carmel Experimental Farm includes 132 10-foot plots divided into four replicates, one replicate of 33 plots in each quadrant.

4. The same pipes may be used for both irrigation and sewage through the use of a simple valve and plug arrangement.

5. Sprays are applied with either a three-nozzle, hand-held spraying device or with the Hayes Jr. sprayer.

6. Valves are arranged at the spray tank so that a preparation may be mixed in one tank while spraying from the other.

7. The spray-rig is equipped with a revolving, overhead boom which carries the spray hose above the plants and out into the plots.

8. Crops which have been used successfully in this design are snap-beans, celery, tomatoes, potatoes and roses.

9. Analyses of variance are presented for four different sets of data demonstrating highly significant treatment effects, even when significant block effects are obtained.

10. A dosage vs. spray-interval interaction experiment is given as an example of the type and precision of information obtainable with this spiral design.

11. The accuracy of the spiral design arises from: (1) relief from tedium and (2) reduction of heterogeneity.

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