THE EFFECT OF CERTAIN SPRAY MATERIALS ON THE TRANSPIRATION OF STRAWBERRY AND APPLE FOLIAGE

by

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INTRODUCTION

Transpiration is the loss of water in the form of vapor from the stem and leaves of the living plant. This phenomenon has been widely studied as a basis for both fundamental and applied research. Many of these studies have concerned the effects of chemicals when applied as sprays on this loss of water from plants.

Miller (1938) considered 1914 as the dividing line between mere observations on the effects of spray materials on transpiration and detailed experiments conducted exclusively to test these effects. He cites the work of Dugger and Cooley (1914) as outlining a method of procedure that has been followed generally by other workers on this problem.

Although the effects of numerous spray materials on transpiration have been tested, by far the largest amount of work has been done with an inorganic fungicide, Bordeaux mixture. The results of these experiments, as well as the conclusions drawn from them, have been somewhat contradictory. Nevertheless the work with Bordeaux mixture has served as a basis for studies on the newer spray materials.

During recent years the use of organic insecticides and fungicides has attracted widespread interest. Outstanding among these materials is the comparatively new insecticide, DDT, (dichloro-diphenyl-trichloroethane). The amount of publicity given to this new material during and following the recent war has been enormous. Research work concerning this new material has barely kept pace with its growing use in the agricultural field.

Possibly not so much publicized but fully as rapid in their rise to popularity are the new organic fungicides. Not only have some of these chemicals given good control against diseases formerly combated by the use of older inorganic materials but they have been found to be effective in the control of certain fungous plant diseases not previously amenable to control by sprays. As is true with DDT, the research work on these materials has been restricted largely to their effectiveness in the control of pests and has not covered their possible secondary influences on plant functions and growth.

The effect of a spray material on transpiration is one of these secondary influences. It was with this in mind that tests were begun in the spring of 1946 to determine the effects of a few of the new organic materials on the transpiration of apple and strawberry foliage and to compare their effects with that of the much studied material, Bordeaux mixture.

The organic sprays tested were Deenate W-50, Fermate, and Zerlate. These three materials are manufactured by and were supplied with the compliments of the Grasselli Chemicals Department of the E. I. duPont de Nemours Company, Wilmington, Delaware. Deenate W-50 is a 50 percent water dispersible, DDT. Fermate and Zerlate are fungicides and are the iron and zinc derivatives of dimethyl-dithiocarbamate.

The experiments were conducted with strawberry plants under greenhouse culture and with two-year-old apple trees growing in the open. These plants are well adapted to the methods of transpiration measurement used and are plants to which the four materials tested are applied in commercial fruit production. Under the conditions of the experiments, however, these sprays were applied to test only their effects on transpiration; no insect or disease pest was involved.

REVIEW OF LITERATURE

The economic importance of transpiration from crop plants, especially in areas of deficient moisture supply, has warranted the extensive amount of research which has been applied to this subject. It is difficult to visualize the amount of water transpired from growing plants. Loomis and Shull (1937) estimate that the water lost by transpiration from one acre of vigorous corn in three months would supply the needs of a city family of four persons for six years. Gardner (1942) states that the amount of water needed to produce 300 bushels of apples and the normal plant growth of one acre of apple trees is equivalent to six to seven inches of rainfall. All but a very small portion of this water is lost from the trees by transpiration.

This transpiration process has been credited with a number of "functions". The more popular of these, the increase in the rate of absorption of nutrients from the soil and the cooling effect on the leaves, have been found to be of minor importance, Loomis and Shull (1937) and Miller (1938). In reviewing this subject, Curtis (1926) concluded that, although transpiration may in many cases actually be harmful, it cannot be eliminated if the plant continues to carry on the essential process of photosynthesis. For photosynthesis the moist cell surfaces of the leaf must be exposed to the atmosphere to allow for the absorption of carbon dioxide and the elimination of oxygen, and wherever moist cell surfaces are exposed to the atmosphere transpiration must necessarily occur.

Factors Affecting Transpiration

If this loss of water from plants is taken as an inevitable occurrence, then the factors which affect the amount of this loss should be considered. Miller (1938) lists six general factors that influence the rate of transpiration; leaf structure, incipient drying and wilting, aerial and meteorological conditions, water content of the soil, chemicals, and fungous diseases. No attempt will be made in this review to cover the vast amount of literature pertaining to the influence of these factors, except to cite work which bears directly on the specific problem at hand.

Leaf Structure. Livingston (1938) gives five "internal influences" that affect transpiration from plant leaves. They are: (1) area of leaf surface, (2) water-vapor pressure of the cuticle, (3) frequency of the stomata, (4) average size of the stomata, and (5) vapor tension in the substomatal chambers.

The importance of the cuticle as a protective coat to prevent the epidermal cells of a plant from too rapid loss of water is stressed by Eames and MacDaniels (1925). However, the mere presence of a heavy cuticle does not necessarily lead to drouth resistance. It has been shown by Swanson (1943) that the American holly having a thick cuticle has, under the same conditions of soil and atmosphere, an average rate of transpiration which exceeds that of either tobacco or yellow coleus, both with thin cuticles.

Epidermal hairs also function in the reduction of water loss from plant parts, Eames and MacDaniels (1935), but again it has been shown that a coating of leaf hairs does not necessarily indicate a reduced transpiration rate from leaves bearing these epidermal outgrowths, Miller (1938). It should be remembered that the leaves of the strawberry are covered by an abundance of plant hairs.

Darrow and Dewey (1934) made stomatal counts on a number of strawberry varieties and found a variation of 200 to 500 per square millimeter of leaf surface on the under side of the leaves. None was found on the upper leaf surface. The stomata of the variety Blakemore were found to be more sunken than those of the less drouth resistant varieties studied. These two workers also found that, contrary to the general conclusions of Livingston (1938) and Maximov (1929), certain varieties with larger and more numerous stomata were better able to endure drouth than other varieties having smaller and less numerous stomata.

Lloyd (1908) found evidence that the stomata are nonregulatory with reference to transpiration. These observations were later corrected by Loftfield (1921) who pointed out that the stomata do influence transpiration but, until they are 50 percent or more closed, their influence is overshadowed by environmental conditions. The latter worker divided the plants he studied into three general groups as to daily stomatal behavior; the barley or cereal type, the alfalfa type and the potato type. The strawberry and apple belong to the alfalfa type which is characterized by stomata which open with the appearance of daylight, close during the day if conditions are unfavorable for the plant and then open for a period at night. Darrow and Dewey (1934) observed that under greenhouse conditions the stomata of the strawberry open to their fullest capacity between the hours of 9:00 and 10:00 a.m. Pickett (1937) found that during the abnormally dry and hot seasons of 1933 and 1934 the stomata of outdoor apple foliage were seldom open after 9:00 a.m. and frequently were closed or nearly so by 7:00 or 8:00 a.m.

That stomatal movement is not entirely due to environmental conditions was demonstrated by Montermoso and Davis (1942), who found that Coleus blumei showed rhythmic fluctuations in transpiration when placed under constant conditions of darkness, temperature, and humidity. They added that the diurnal maximum and minimum fluctuations of transpiration could be reversed by subjecting the plants to reversed light and dark periods using artificial light.

Stomata on apple leaves are found only on the lower surfaces. Pickett (1937) made stomatal counts on orchard-grown apple foliage and found that the Winesap variety had an average number of 330 stomata per square millimeter of leaf surface. Eames and MacDaniels (1925) give 250 stomata per square millimeter of leaf surface as an average for all apple varieties.

Turrel (1936) showed that a xeromorphic leaf may have a higher ratio of exposed mesophyll to epidermal surface than a mesomorphic leaf, and suggested that the greater rate of transpiration demonstrated by many xeromorphic species may be accounted for in these terms. The same worker, in 1944, working with the mesomorphic plant, periwinkle, and the xeromorphic plant, oleander, found the ratio of internal-external exposed surface to be greater for the xeromorphic plant with a corresponding increase in the rate of transpiration per unit of area.

Parija and Samantarai (1939) measured the rate of transpiration from leaves of the plants Datura alba and Helianthus annus from the first measurable stage in leaf growth to leaf fall. They found that as young leaves expand in size the rate of transpiration rises rapidly, then falls off to a steady value for the greater period of the leaf's activity, after which it declines.

Aerial and Meteorological Conditions. Livingston (1938) lists radiant energy, air temperature, relative humidity, and air movement as the "external factors" influencing transpiration. Nutman (1941), using a weighing device whereby the transpiration rate of large plants could be determined over short periods of time, found that under the conditions of his experiment the daily march of transpiration from a Coffea arabica tree is due almost entirely to variations in the incident radiation. Nutman adds that transpiration may be influenced by both radiant energy and saturation deficit, independently of temperature.

Using the plants Helianthus annus and Ambrosia trifida, Martin (1943) determined that the correlation of relative humidity to water loss was linear at temperatures of 27, 38 and 49 degrees centigrade. The influence of wind on the rate of transpiration increased considerably with an increase in temperature. Martin considered that a possible explanation of this increase is because of a corresponding increase in the cuticular component of transpiration.

Thut (1938) attempted to measure the effect of relative humidity alone on water loss. He used "humidity bottles" containing various concentrations of sulfuric acid which he attached to the leaves of coleus, hibiscus, cotton and other plants. The relative humidity inside the bottles was varied by varying the concentration of the acid. The water loss from the leaves of these plants was an inverse linear function of the relative humidity over the entire range from 100 percent to nearly zero relative humidity. These plants ranged from 13.2 to 17.6 units of water lost for every 10 percent decrease in relative humidity. Thut suggested that the point where transpiration ceases due to high relative humidity is the relative humidity of the intercellular spaces of the leaves.

Briggs and Shantz (1916) correlated various weather conditions to the transpiration rate of small grain crops. They found a correlation coefficient of +0.77 between wet bulb depression and transpiration, +0.60 for temperature, +0.42 for radiation and +0.05 for wind. They explained the fact that these figures add to more than unity by suggesting that there is a marked interaction between these factors. These workers (1916) also show that under natural conditions favoring high evaporation plants do not respond wholly as a free evaporating surface even if "bountifully" supplied with water and when no visible wilting occurs. They found (1917) a wide departure of the hourly transpiration of alfalfa leaves and evaporation from a porous cup atmometer. They explained this departure on the basis of (1) the evaporation over the transpiration at night, (2) the more pronounced effect of wind on atmometers, and (3) the lesser effect of solar radiation on atmometers. However, they pointed out that there was a close correlation of daily transpiration to evaporation.

Water Content and Temperature of the Soil. The necessity for an ample amount of moisture in the soil to maintain turgidity of the cells of the plants thereon is evident for a transpirational study. Eaton (1929) measured the transpiration rates of turgid and wilted leaves of cotton and found a correlation coefficient of +0.929 ±0.025 between differences in leaf temperature and differences in transpiration rates of leaves in these two conditions. He found the maximum mean difference in the temperature of turgid and wilted leaves to be 5.5 degrees centigrade. Schneider and Childers (1941) pointed out that reduction of soil moisture reduced transpiration from apple leaves as much as 65 percent of normal before wilting occurred. After wilting, transpiration was reduced to 87 percent and, after watering, two to seven days were required for the trees to reach normal transpiration rates, depending upon the severity of the wilting.

On the other hand, exceedingly high soil moisture levels can cause a decline of transpiration, Foster and Tatman (1940). Gray (1939), working with strawberry plants, found that variation of soil moisture between 40 and 80 percent of the water holding capacity of the soil did not affect transpiration. In 1941 he observed that root development is retarded and small roots injured at soil temperatures of 100 degrees Fahrenheit. Kozlowski (1943) determined the effect of low soil temperatures on transpiration from loblolly pine and eastern white pine during the dormant season. He found that a decrease of soil temperature from 30 to zero degrees centigrade produced a correlated decrease in the rate of transpiration.

Chemicals. Transpiration from plants may be affected by chemicals when they are applied to either the soil or the foliage. Felber and Gardner (1943) have shown that methylcellulose when added to the soil reduced transpiration from potted plants 30 to 50 percent.

Much work has been done in recent years on the influence of waxes and oils in the reduction of the water loss from plants. Spray materials applied to plants for the purpose of combating disease and insect pests may likewise affect transpiration. By far the greater portion of the work to test this plant response to chemicals has been done with the fungicide, Bordeaux mixture. So extensive is the work with this material that Horsfall and

Harrison (1939) suggest the term "Bordeaux transpiration" to abbreviate the phrase "the effect of Bordeaux mixture on the transpiration from plants".

Some of the early workers considered that Bordeaux mixture decreased transpiration. Later it was concluded that it accelerated transpiration. Some workers have found that Bordeaux mixture gives little or no increase in transpiration. These differences are probably best explained by the differences of the conditions under which these tests were made.

Southwick and Childers (1941) measured the transpiration from Stayman Winesap apple leaves under constant illumination and found that water loss was uninfluenced or somewhat depressed by Bordeaux mixture and its component parts. Childers (1936) had reported previously that with mature tomato plants a 3-4-50 Bordeaux mixture had no effect on transpiration under conditions of either high or low water loss. Foster and Tatman (1940) also found that the rate of transpiration was not influenced by this material; nor could these workers detect any interaction between the environmental conditions and the effect of the spray. Miller and Schuster (1945) found no effect of either high- or low-lime Bordeaux mixture on Persian walnuts and filberts under the conditions of their experiments. Loustalot (1944) reported that as many as three applications of this fungicide to pecan leaves had no effect on water loss.

Nevertheless, the effect of Bordeaux mixture on the water lost from plants is of economic importance. Gardner, Bradford, and Hooker (1939) state that cherries from trees sprayed with

Bordeaux mixture are much smaller in size than those from unsprayed trees owing to the water deficit within the trees occasioned by the higher transpiration rates caused by the copper compounds. Wilson and Runnels (1931-1938) started their many experiments on the effect of Bordeaux on water loss with the observation that ginseng, Panax quinquefolium L, when sprayed with this material suffered severely from drouth and in some cases was so injured that the plants were not able to recover.

Hedrick (1907) observed that wet weather at spray time increased plant injury due to Bordeaux mixture. It was noted by Horsfall and Harrison (1939) that high temperatures at spray time favored "Bordeaux transpiration" but high temperatures thereafter had little effect.

Those workers who have found Bordeaux mixture to increase water loss are not agreed as to the cause of this influence. Duggar and Bonns (1918) concluded that this increase was due to the passage of moisture over the Bordeaux film, the increase being more apparent at night. Wilson and Runnels (1933) report that the increases they found with this material are due to increased cuticular transpiration. Krausche and Gilbert (1937) also have found evidence to show that the increased transpiration observed is not stomatal and that copper sprays do not affect the behavior of the stomata. According to the findings of Martin (1916), copper sulfate dusts have less effect on transpiration than does a film of Bordeaux mixture. Martin and Clark (1929) found that a second application of this spray material did not result in an added increase in transpiration, but spraying with water after two applications did result in a second increase, possibly due to a renewal of the Bordeaux film.

That inert dust of Bancroft clay and silica increase night-time transpiration was observed by Wagner (1939). This effect was later studied by Beasley (1942). She found that fine dusts of Bancroft clay and talc applied in the daytime on the stomata bearing surface increased the transpiration at night. She could find no effect of these materials on plants in the sun. Coarse talc and silica had less influence than did the finer dusts. This worker reasons that the dust particles prevent complete closure of the stomata at night, so causing increased water loss at this period of the day, but that there would be little effect on plants in the field.

The effect of the color of Bordeaux mixture residue on transpiration was tested by Wilson and Runnels (1938). They found that Bordeaux mixture reduces leaf temperatures over untreated leaves and may reduce transpiration in the daylight hours. However, when lampblack was added to the spray material the temperature of the sprayed leaves rose above that of the surrounding air and the unsprayed controls.

A tentative hypothesis of Bordeaux induced transpiration is given by Horsfall and Harrison (1939).

> The alkalinity of Bordeaux mixture saponifies the cuticle, however it may clog some stomata. It may cool the leaf in bright weather. The balance between these various factors will determine the magnitude of Bordeaux transpiration. In the field, where cuticles are thick and hard, it may be true at times that the effect on the cuticle may be so slight as to be overbalanced by the factors of reduced water loss.

As yet very little is known as to the influence of DDT and the new organic fungicides on plant activity. Fortune magazine (1946) gives a review of the vast amount of popularized material that has been published pertaining to DDT. It has been demonstrated that this organic insecticide is toxic to some plants. Cucurbits are killed or stunted by small dosages of this material. Tomatoes, corn, beans, and rye are sometimes retarded.

Moon and Harley (1946) observed that apple leaves sprayed with DDT appear normal in every way, but add the caution that the absence of a visible injury is not conclusive evidence that leaf function may not be affected to some degree. These workers, however, found no influence of DDT on the efficiency of apple leaves in terms of fruit growth rates.

Bailey and Sproston (1946), in tests with Fermate on the cultivated blueberry, could not detect any injury to either fruit or leaves from the use of this material. Although some work has been done on the use of Fermate in the treatment of seeds, lily bulbs, and woody and herbacious cuttings, White (1946) observed that Fermate alone does not stimulate rooting. However, when geranium cuttings were treated with combinations of Fermate and certain growth substances these cuttings developed more and longer roots.

Methods of Measuring Transpiration

Miller (1938) describes the four universal methods of transpiration measurement. These are the potometer method, the weighing method, the cobalt chloride-paper method, and the

Freeman method. There is no need to repeat the description of these methods in this paper, only to point out some of the points that were considered in choosing the two methods used.

Glover (1941) set up four rules that, if possible, should be fulfilled by a method for measuring transpiration:

> (1) The part of the plant being studied should be attached to an intact rooted plant. (2) The method adopted would not alter the environmental conditions. (3) The transpiration rate should be calculated in absolute units, i.e. weight of water/unit area/unit time. (4) The transpiration rate should be measurable over short intervals of time, preferably continuously.

The potometer method of measuring water loss, in which cut leaves or stems are placed in containers of water, has been severely criticized by Loftfield (1921) and Maximov (1929). The former worker found that the stomatal movement of cut stems differed greatly from that found in potted plants and field plants. Gray (1939 and 1941) modified the usual potometer method by immersing the roots of strawberry plants in flasks of water, but even with this modification he could not obtain results with these plants that corresponded with those obtained from plants growing in soil.

Miller (1938) and Maximov (1929) have both shown a preference for the weighing method. Briggs and Shantz (1915) describe in detail a number of automatic transpiration balances of large capacity that may be used in the field.

The Freeman method of measuring the rate of transpiration as modified by Heinicke and Hoffman (1933) has been widely used. The transpiration chamber, the rate of air flow, and the drying agents used have been changed by various workers but the method remains much the same as originally described. Loustalot and Hamilton (1941) found that cellophane envelopes used as transpiration chambers proved unsatisfactory because the leaf tissues were injured by high temperatures inside the envelopes on prolonged exposure to sunlight. These workers also found it advisable to increase the rate of air flow through the leaf containers from 100 liters per hour, as originally used by Heinicke and Hoffman to 2 to 2.5 liters per square centimeter of leaf surface tested per hour.

The desiccant, calcium chloride, was used in the original Freeman, and Heinicke and Hoffman methods to absorb the moisture transpired. Other workers have used phosphorus pentoxide, calcium sulfate, and pumice stone impregnated with sulfuric acid, Loustalot (1944). Morton (1938), in stressing the importance of choosing the correct drying agents for a double desiccant arrangement, points out that it would be ludicrous to attempt to dry air by initial scrubbing with sulfuric acid followed by passing it through a less effective calcium chloride, although such errors have been reported in formal publications.

METHODS AND MATERIALS

For a quantitative measurement of transpiration, the weighing method is probably the most convenient and satisfactory. Nevertheless, this method has some distinct limitations. Since the soil and the container in which the plants are grown must be weighed, this method is best adapted to plants which require a minimum amount of soil for normal root growth. Also, experiments using this method of transpiration measurement are usually conducted in a greenhouse or under some type of shelter where the plants can be protected from rain. Single strawberry plants were chosen as being adaptable to this system of measurement.

It is evident that greenhouse conditions are not comparable to those in the field. Therefore, some system of measuring the water loss from field grown plants, preferably of a deciduous fruit tree, was desired. The Freeman method as modified by Heinicke and Hoffman was selected. Two-year-old apple trees of the variety Winesap were used in this portion of the experiment.

The experiments fall very naturally into two sections: that dealing with the effects of the spray materials tested on the transpiration of (1) strawberry plants in the greenhouse and (2) the apple trees growing in the field. The same spray materials were tested in both locations.

Strawberry Experiments

Dates of the Experiments. These experiments covered two consecutive twenty-day periods: April 26 to May 17 and May 17 to June 6, 1946. With only minor changes, which will be explained later, the two tests were conducted similarly and are herein spoken of as Run Number 1 and Run Number 2.

Plant Materials. One month prior to the start of these experiments 100 runner strawberry plants of the variety Blakemore (Yellows free) were moved from a field plot to the greenhouse. These plants were placed in five-inch unglazed clay pots, using a soil mixture of three parts potting soil to one part sand. The plants were pruned to three or four leaves per plant. The plants were well established by the time Run Number 1 was begun.

These plants seemingly were free from all disease and insect pests. One very light infestation of strawberry leaf roller was brought under control before the tests were begun by removing the larvae from the affected plants. However, during Run Number 2 an abnormal unfolding of the new leaflets occurred which could not be traced to any known causative factor. The injury appeared as an inability of the new leaflets to unfold properly at their tips and caused considerable deformity as the leaves matured (Plate I, Figs. 1 and 2). What effect this had upon the rate of transpiration from these leaflets is not known, but since it occurred rather uniformly on all plants, it was hoped that the effect was comparable throughout the experiment.

<u>Plant Containers</u>. Inexpensive plant containers were made from Number 10 (near gallon) tin cans. The unglazed pots in which the plants were grown were lowered into these cans onto blocks that held the top of the clay pots to a level one-fourth inch above the top of the cans. The space between the surface of the potting soil and the top of the pots was partially filled with coarse sand (Plate II, Fig. 4 and Plate III, Fig. 5).

The potted plants were sealed in the can with waterproof oilcloth (Waltex) covers. Two holes, each one-half inch in diameter, were cut in the covers with a cork borer prior to the sealing of the cans. One hole allowed for fitting the covers around the crown of the plants and the second hole was used for the addition of water to the pot. The covers were placed around

EXPLANATION OF PLATE I

Fig. 1. Abnormal unfolding of the new leaflets that occurred during Run Number 2 of the strawberry experiments as it appeared on a potted plant.

Fig. 2. This abnormal leaf condition on immature and fully developed leaflets.

PLATE I

Fig. 1.

Fig. 2.

EXPLANATION OF PLATE II

Fig. 3. A typical plant sealed in the manner used in the strawberry experiments.

Fig. 4. A five-inch unglazed clay pot as set in a Number 10 tin can and the Waltex covers used to seal the plants in the strawberry experiments.

Fig. 3.

Fig. 4.

EXPLANATION OF PLATE III

Fig. 5. A typical plant growing in a five-inch unglazed pot as used in the strawberry experiments. Note the layer of sand covering the surface of the potting soil.

Fig. 6. A representative plant showing good root distribution on removal from the sealed container in which it had been growing for a period of 20 days.

PLATE III

Fig. 5.

Fig. 6.

the plants by slitting these covers from one side to the center hole and later sealing with waterproof tape. Plate II, Fig. 3 shows a typical plant sealed in this manner.

Water added to the pots in the course of the experiment spread through the sand layer and uniformly into the soil below. The space inside the cans around the pot provided for aeration. At the close of each run, the plants were inspected and found to have their roots well distributed throughout the soil mass indicating that the distribution of water and the aeration were both satisfactory (Plate III, Fig. 6).

During Run Number 2, the weight loss of a blank container was recorded. This blank consisted of a pot sealed in a container as described above. The center hole of the cover was closed with a cork. The pot contained soil with the same moisture level as that in which the plants in this run were growing. During the twenty-day period of the run this blank container lost 19 grams or 2.6 percent of the total weight loss of the plant having the least water loss in the experiment.

Weighing. The plants were weighed daily and water added every second day to restore them to their original weight. The weighing was done between six and eight a.m. throughout the experiments. A torsion balance with an accuracy of ±1 gram was used for these measurements.

Spraying and Spray Materials. Forty plants were used in each run. The plants, growing in their sealed containers, were placed in an empty greenhouse bench as shown in Plate IV, Figs. 7 and 8. The daily weight losses were recorded for a ten-day

EXPLANATION OF PLATE IV

Fig. 7. Arrangement of plants in an empty greenhouse bench for the strawberry experiments. Looking down the bench.

Fig. 8. Arrangement of plants in an empty greenhouse bench for the strawberry experiments. Looking across the bench.

PLATE IV

Fig. 7.

Fig. 8.

period prior to spraying. The plants were then divided into five groups, eight plants each selected at random. The plants in each group were sprayed with one of the four spray materials under test, the remaining eight plants serving as controls. Weight losses were then recorded for a second ten-day period.

The spray materials were made up in one-gallon quantities; distilled water was used in all cases. The three organic materials, Fermate, Zerlate, and Deenate W-50, were mixed at the rate of two pounds per 100 gallons of water. This is the maximum concentration recommended by the manufacturer. The Bordeaux mixture was mixed at the rate of four pounds copper sulfate and eight pounds fresh hydrated lime per 100 gallons of water. These materials were freshly mixed just before spraying. The Bordeaux mixture was prepared by adding the copper sulfate, in solution, to three-fourths gallon of water, stirring into this a paste slurry of lime and water, and making to one gallon.

One-half teaspoonful of Grasselli Spreader and Sticker was added to each gallon of the freshly mixed materials. Deenate W-50 is a water dispersible powder and could have been used without a spreader. The other three materials, however, do not spread well over the hairy foliage of the strawberry and the above amount of spreader and sticker was found to cause good coverage with all of the materials without causing excessive run-off. The control plants were sprayed with distilled water.

The spraying was done with a paint-gun type atomizer. The leaves were thoroughly wetted on both sides. In both runs the spraying was done between 8:00 and 10:00 a.m., immediately following the tenth weighing of the plants. On May 6, 1946, the date Run Number 1 was sprayed, the weather was dull and cloudy and the greenhouse temperature between these hours remained at 57 degrees Fahrenheit. On May 27, the day Run Number 2 was sprayed, the weather was bright and sunny and the greenhouse temperature rose from 63 degrees Fahrenheit at 8:00 a.m. to 70 degrees Fahrenheit at 10:00 a.m.

Additional Records and Measurements. A record was kept of the daily loss of water from a spherical porus cup atmometer. The atmometer was connected to a burette, (Plate V, Fig. 9), in much the same fashion as described by Miller (1938).

A continuous record of the bench level temperatures was recorded by a thermograph throughout the experiment.

At the conclusion of each run the leaves from all plants were removed, blueprinted, and the image areas measured with a planimeter. In addition to this final leaf measurement, the length and width of the leaflets were taken at the time the plants in Run Number 2 were sealed and at five-day intervals thereafter. The products of these leaflet length x width measurements were later correlated with leaf areas as measured with a planimeter.

At the beginning of each run, soil samples were taken and the average soil moisture, as a percent of the water holding capacity, determined.

EXPLANATION OF PLATE V

Fig. 9. A porous cup atmometer attached to a burette as used in the strawberry experiments.

PLATE V

Fig. 9.

Apple Tree Experiments

Dates of the Experiments. These tests were conducted throughout the summer of 1946. They were divided into three parts. Part I covered the period from May 31 to July 11 and was designed to test the effects of successive spray applications on the rate of transpiration. Part II was conducted during the weeks of July 25 to July 30, August 5 to August 10, and August 16 to August 21. This part of the experiment was set up to test the effects of single applications of the various materials when applied to separate branches of the same tree. Part III of the tests was run to determine the effects of the spray materials tested upon the rate of transpiration at various times of the 24-hour day. These latter tests were conducted August 21, 22, and 23.

Plant Materials. These tests were conducted on 30 two-year-old Winesap trees growing at the south end of the irrigated garden on the Kansas State College campus. The trees were planted March 15, 1946, and were well established before the tests were begun. They were arranged in two rows, and spaced four feet between rows and three and one-half feet apart within the rows. The area was nearly level, clean cultivated, and had been uniformly cropped the year before. All of the trees were healthy and vigorous although there was a noticeable lack of uniformity in their growth rates. Spider mite infestation was severe on the Deenate W-50 sprayed trees late in the season but did not show up during the course of the experiments.

Method of Transpiration Measurement. The Freeman method basically embodies the drawing of a known quantity of air through a transpiration chamber containing the leaves under experiment, through U-tubes containing a drying agent, and measuring the increase in weight of the tubes over the increase of a duplicate set of tubes used for normal air. The glass cylinder transpiration chamber used by Freeman was replaced with a cellophane envelope by Heinicke and Hoffman. The method used in the experiments covered by this portion of this paper was an adaptation of the method outlined by the latter workers.

A cart (as shown in Plate VI, Fig. 10) was devised in which the necessary apparatus needed in the conduct of these experiments could be moved from the laboratory to the field. Since the effects of four spray materials were being tested against unsprayed foliage, a minimum of six separate air lines was required. The sixth line was necessary to determine the amount of moisture in normal air. Each air line consisted of a five-foot piece of rubber tubing extending from the cellophane leaf envelopes, to U-tubes connected in series and a flometer (Plate VI, fig. 11).

The cellophane leaf envelopes were those used as liners in frozen food containers, one pint capacity, and enclosed four fully developed leaves. (The method of selection of these leaves will be explained in the discussion of the various parts of these experiments.)

The first of the U-tubes contained anhydrous calcium chloride and the second, anhydrous calcium sulfate (Drierite).

EXPLANATION OF PLATE VI

Fig. 10. The apparatus used for determining the water loss from the foliage of trees in the field as used in the apple tree experiments.

Fig. 11. A diagram of a single air line of the above apparatus. (1) Rubber tubing to connect the cellophane leaf envelope to the U-tubes. (2) Calcium chloride tube. (3) Calcium sulfate (Drierite) tube. Note corks used in these tubes while weighing. (4) Flometer. (5) Capillary-tube constriction. (6) Safety reservoir. (7) Connection to the aspirators.

PLATE VI

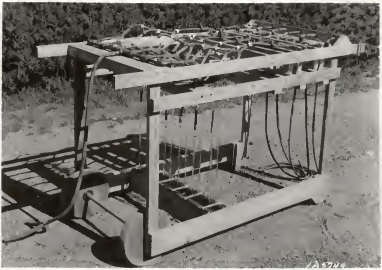

Fig. 10.

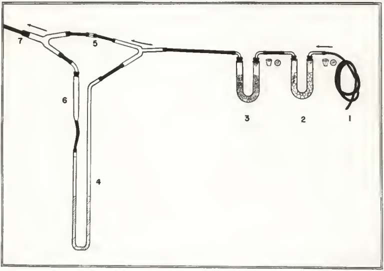

Fig. 11.

The flometers were made of two-foot U's of glass tubing filled one-third full with water over which a shunt containing a capillary tube was attached. These flometers were calibrated with a wet-test flometer to pass 100 liters of air per hour. The six lines were connected to two aspirators working in parallel.

Calcium chloride (4-mesh) was chosen for the first U-tube in the line since it will absorb a relatively large amount of moisture before changing form and retarding the flow of air. This material was discarded at the close of each run. The Drierite (8-mesh) used in the second U-tube is manufactured by W. A. Hammond Drierite Company of Xenia, Ohio. Although this material will not take up as much moisture as will an equal volume of calcium chloride, it is the more efficient drying agent. This material was regenerated at the close of each run by heating at 215 to 235 degrees centigrade in a muffle furnace for a one-hour period. Non-indicating Drierite was used in these experiments. Indicating Drierite may be obtained at a higher cost and, although it is more convenient than the non-indicating type, it is no more efficient. Plain six-inch U-tubes were used due to the cost of the Schwartz ground-in-stopper type.

The steps in an individual run proceeded as follows: The U-tubes were filled approximately three-fourths full with the drying materials and immediately closed with corks. These corks were numbered corresponding to numbers placed on the U-tubes. The weight of the closed tubes and dry desiccant was recorded. The weighing was done on a small torsion balance to an accuracy of ±1 milligram. The weight of the leaf envelopes was also recorded. The tubes were then uncorked and placed as quickly as possible in the line. These lines were closed on both sides of the drying tubes with pinch clamps. The apparatus was then taken to the field.

The leaf envelopes were placed over the selected foliage and the connecting tubing adjusted in the bags. The envelopes were fastened in place with paper clips as shown in Plate VII, Figs. 12 and 13. The pinch clamps were then removed and the water to the aspirators turned on. By controlling the flow of water through the aspirators and by the use of adjustable pinch clamps on the individual lines, the flow of air through the lines was regulated to the desired rate. The time between placing the envelopes on the leaves and the time the flow of air was regulated was kept to a minimum. The tubes were numbered to correspond with the number of the tree or branch from which the air lines extended.

At the end of the run the lines were closed with the pinch clamps, the leaf envelopes removed and folded so that any moisture collected in them was retained, and the apparatus returned to the laboratory. The U-tubes were removed from the lines, the corks replaced in their respective tubes, and the weights of the tubes and the used desiccant recorded. The increase in weight of the tubes and desiccant plus the increase in weight of the leaf envelopes represented the amount of moisture transpired by the leaves and the amount in normal air. By subtracting from this total the increase in weight of the

EXPLANATION OF PLATE VII

Fig. 12. A leaf envelope enclosing four mature leaves as used in Part I of the apple tree experiments.

Fig. 13. A leaf envelope enclosing the four upper leaves of a previously pruned shoot as used in Parts II and III of the apple tree experiments.

PLATE VII

Fig. 12.

Fig. 13.

desiccant in the set of tubes absorbing only that moisture present in normal air, the absolute amount of water lost by the foliage was obtained.

To test the consistency of the apparatus, a number of runs were conducted to determine if, when each line measured only the moisture normally in the air, the amount of moisture picked up in the lines would be similar. The greatest variation within such a test amounted to 2.5 percent of the weight increase of the least efficient line.

Schedule of the Experiments. As has been noted before, the apple tree experiments were conducted in three parts. These parts differed in objective and procedure although the same spray materials were tested.

Part I. This portion of the experiments was designed not only to test the effects of the spray materials upon transpiration but also to indicate the effects of the spray residue resulting from as many as six spray applications.

The 30 trees were divided into six groups of five trees each. One tree, selected at random, within each group was sprayed with each material tested and the fifth tree sprayed with water and used as a control. Transpiration tests were run on the five trees in each group simultaneously, the order of testing between groups being determined by chance. The trees were sprayed at weekly intervals. For two days prior to spraying and the first, third, and fifth days following spraying, transpiration tests were run on the group selected. Group Number 1 represented the effects of the first spray application, group

Number 2 that of the second application and so on for the six groups. Thus, four selected leaves on each tree were tested five times during the seven day period alloted to each group of trees. At the end of this time these leaves were removed and blueprinted.

The transpiration tests covered a one-hour period between 9:30 a.m. and 12:30 p.m. The spraying was so timed that the first test following the spray application could be run as soon as the surface of the foliage was dry.

At the time of the first spray application, the youngest fully-opened leaf on each shoot was tagged in order that leaves for the individual tests could be selected which were of approximately the same age and had received the same number of spray applications. The leaves chosen for use in this part of the experiments were similarly located, all on shoots arising from the first scaffold branch on the north side of the trees. The leaf envelopes were opened at both ends and slipped over the shoots as shown in Plate VII, Fig. 12. The rubber tubing extending from these envelopes to the drying tubes was so adjusted that its open end reached approximately to the center of the envelopes. As air was drawn through the tubing it was replaced by air entering both the upper and lower ends of the envelopes. Plate VIII, Fig. 14 is a photograph of the apparatus as it was used in these runs.

Part II. As the test in Part I progressed, it became evident that the tree to tree variation was materially affecting the results obtained. In order to reduce this portion of the

EXPLANATION OF PLATE VIII

Fig. 14. The apparatus used for determining the water loss from the foliage of adjacent trees in the field as it was set up for Part I of the apple tree experiments.

Fig. 15. The apparatus as it was used to determine the water loss from the foliage of separate branches of the same tree, Parts II and III of the apple tree experiments.

PLATE VIII

Fig. 14.

Fig. 15.

experimental error, a series of experiments were designed whereby it was possible to test the effects of these four spray materials when applied to separate branches of the same tree; the assumption being that there would be less normal branch to branch variation than tree to tree variation.

Three of the unsprayed control trees of the former experiments were used in these tests. Five shoots on each tree were selected, one for each spray material tested and one as an unsprayed control. The shoot sprayed with each material was selected at random. These shoots were located at approximately the same level on each tree.

In order to avoid slitting the bottom of the leaf envelopes and still enclose only four fully developed leaves, that portion of the shoots above these leaves was removed prior to making the tests. Plate VII, Fig. 13 shows a leaf envelope placed over four leaves and the connecting tubing in place. The transpiration tests were run two days prior to spraying and four days following spraying. These tests covered a two-hour period between 8:00 and 11:00 a.m. In order to duplicate the tests, the effect of only one spray application was tested on each tree. Plate VIII, Fig. 15 shows the apparatus as it was set up for these tests.

Part III. Since the preceding tests gave measurements of the transpiration only between 8:00 a.m. and 12:30 p.m., it was thought advisable to conduct a few runs that might indicate the effects of the spray materials under test on the water loss at other periods of the day. A fourth unsprayed control tree was used in these tests. Individual branches were sprayed as in Part II with three weekly spray applications. The third spray was applied the forenoon preceding the first transpiration test. These tests were conducted for two consecutive days, each covering a two-hour period between 2:00 and 4:00 p.m., 10:00 and 12:00 p.m. and 6:00 and 8:00 a.m. The leaf envelopes were attached to decapitated shoots as in Part II.

The first two spray applications were both followed within 24 hours by rain but since the spray had fully dried considerable residue remained at the time of the third application.

Spraying and Spray Materials. The spray materials were made up in two-gallon quantities. As in the strawberry experiments, a 4-8-100 Bordeaux mixture was used and the organic materials mixed at the rate of two pounds per 100 gallons of water. The amount of spreader and sticker was reduced to one-fourth teaspoonful per gallon of water. The spray materials were freshly mixed just before each spraying.

The spraying was done with a three-gallon capacity compressed air sprayer. To reduce drift, the trees or branches were enclosed in a three-sided shield at the time of spraying. The foliage was thoroughly wetted at each spray application.

Additional Records and Measurements. During each run of Parts I and II, the relative humidity was determined with a sling-psychrometer. During the two-day period covered by the tests of Part III, a recording hygrograph and thermograph were kept in operation in the area of the experiments. During each run of Parts I and II the air temperatures, both outside and inside the leaf envelopes, were likewise taken. The general weather conditions during each test and at each spray period were recorded.

At various times during the experiments a Lloyd Hygrodeik was used to indicate the absolute amount of moisture in the air. This hygrodeik was calibrated to give the amount of water in grains per cubic foot of air. The amounts recorded were converted to milligrams per liter and compared to the amount of moisture absorbed by the drying agents in the normal air line of the transpiration measuring apparatus. The greatest difference noted between these two measurements amounted to 17.6 percent of the moisture absorbed by the drying agents, the average difference being 8.9 percent.

Following the last test on each set of leaves they were removed, blueprinted, and their image areas measured with a planimeter.

RESULTS AND CONCLUSIONS

Before attempting to discuss the results obtained in these experiments the terms "transpiration", "rate of transpiration", and "water loss" should be defined. Transpiration, in its narrower usage, refers to the process whereby water is lost in the form of a vapor from the plant. Rate of transpiration denotes the amount of water so lost during a given period of time per unit of leaf surface. Water loss, when correctly used, includes (1) the water lost by transpiration, and (2) that lost by guttation. However, since practically all of the water that is lost from a plant escapes in the vaporous form (Miller, 1938), the terms "rate of transpiration" and "rate of water loss" are often used interchangeably. The three terms just defined are also used interchangeably in this paper although the data collected refer in all cases to the rate of transpiration.

Strawberry Experiments

Aside from the fact that the two runs conducted with strawberry plants in the greenhouse were made over consecutive 20-day periods, there were two major differences in the conduct of these runs; namely, the moisture level of the soil and the method of measuring the transpiring surface of the plants. Run Number 1 was conducted with the soil in which the plants were growing kept to an average moisture content of 31.0 percent of its moisture holding capacity. During Run Number 2, this percentage was increased to 55.0. Although there was no noticeable wilting of the plants with the lower moisture level, since the second run covered a later period in the spring with rising greenhouse temperatures, the higher moisture level was intentionally maintained. This increase in the amount of moisture available to the plants probably affected the overall rate of transpiration but as the increase was uniform with all plants of Run Number 2 its effect should have been equal between the treatment groups.

The standard procedure in calculating transpiration rates is to base the water loss on the area of leaf surface at the close of the experiment, the assumption being that any change in this area throughout the experiment is proportional for all plants. The transpiration rates determined for the plants in Run Number 1 were based on this final leaf area.

Horsfall and Harrison (1939) and Bates and Pickett (Unpublished paper) found it desirable to ascertain the leaf areas at regular intervals during the course of their experiments. These areas can be determined by taking the length and width of the leaves and later converting the product of these measurements into accurate estimations of the leaf area. At the close of Run Number 2, the leaf areas of the plants, obtained by planimeter measurements of the blueprinted leaflets, were correlated with the sums of the length x width products of the leaflets. By a correlation analysis an exceedingly high positive correlation was found to exist. The value of r, the correlation coefficient, was +0.996. This high correlation is explained by the fact that it does not represent measurements of single leaflets but of all the leaflets on one plant, the irregularities of an individual leaflet being balanced by opposing irregularities of another.

When plotted on coordinate paper, it was found that the sum of the length x width products to the leaf area is a straight line function. Figure 16 is the scatter diagram obtained when these measurements were so plotted. The value of b, the regression coefficient, was found to be 0.678.

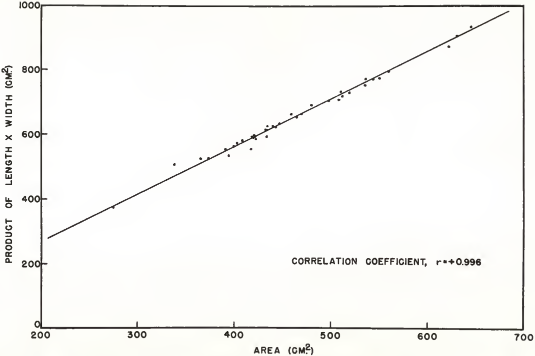

Fig. 16. The scatter diagram obtained by plotting the sum of the products of leaf length x width to plant leaf area, and the regression line that represents this association. (Strawberry experiments, Run Number 2)

By using the formula

$$\hat{X} = \bar{X} + b(Y - \bar{y})$$

where $\hat{X}$ = calculated leaf area
$\bar{X}$ = mean of measured leaf area at the close of the run
b = regression coefficient
Y = sum of leaflet length x width products during the run
$\bar{y}$ = mean of sums of leaflet length x width products at the close of the run

the calculated leaf areas at five-day intervals throughout the run were obtained. A uniform growth rate was assumed for the intervening days. Thus the leaf area of each plant was determined for each day of the run. In obtaining the rate of transpiration per day the calculated leaf area of the day preceding the date of weighing was used since the loss in weight represented the water lost over the preceding 24-hour period.

The added accuracy acquired by calculating the actual transpiring surface throughout the experiment lies in the fact that the plants under test did not make a uniform increase in leaf area. Figure 17 illustrates the varied growth rates of four plants of Run Number 2.

An analysis of variance when applied to the rates of transpiration obtained the first day following spraying indicated that in neither run did the between treatment F ratio of the between treatment variance approach the 0.05 level of probability. Consequently the transpiration rates for the entire ten-day period following spraying were analyzed.

The averages of the rates of transpiration of the spray treatment groups are presented in Table 1. Due to the daily

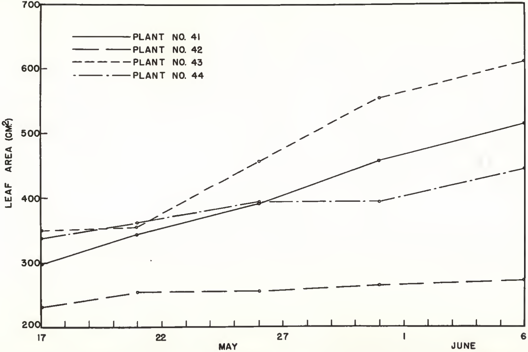

Fig. 17. The varied rates of increase in leaf area of four sample strawberry plants during the 20-day period (May 17 to June 6, 1946) covered by Run Number 2 of the strawberry experiments.

Table 1. Average rates of transpiration for strawberry foliage over the 10-day period before and after spraying; with the treatment:control ratios of these rates and their differences during these periods.

Treatment group	Average rate of transpiration ($Gm./m.^2/day$)		Treatment:control ratio ($\frac{T}{C}$)		
	Before spraying	After spraying	Before spraying	After spraying	Differences
	Run Number 1 (April 26 to May 17, 1946)				
Fermate	754	734	0.959	0.927	- 0.032*
Zerlate	799	724	1.017	0.912	- 0.105
Deenate W-50	824	688	1.048	0.870	- 0.178
Bordeaux mixture	811	750	1.032	0.947	- 0.085
Control	786	792	-----	-----	-------
	Run Number 2 (May 17 to June 6, 1946)				
Fermate	1617	1221	1.124	1.097	- 0.027
Zerlate	1540	1230	1.071	1.105	0.034
Deenate W-50	1537	1103	1.069	0.991	- 0.078
Bordeaux mixture	1616	1268	1.124	1.139	0.015
Control	1438	1113	-----	-----	-------

* A minus value indicates a reduction in the treatment:control ratio after spraying.

changes in light, temperature, and relative humidity it is impossible to compare directly the effects of the spray applications on the water loss. Nor does a direct comparison of the average transpiration rates of the treatment groups take into consideration the differences between the groups before spraying. If, however, the treatment group rates are compared to the transpiration rate of the controls (T:C ratio) both before and after spraying the differences between these two ratios may be taken as an indication of the effects of the spray materials.

By this method of comparing the average transpiration rates, it was found that in Run Number 1 all of the sprays decreased the transpiration when compared with the control plants; the greatest decrease being caused by the Deenate W-50. During Run Number 2 Deenate W-50 was again found to reduce the rate of transpiration. However the Zerlate and Bordeaux mixture sprayed plants showed a slight increase in transpiration due to these sprays when compared with the control plants. It must be remembered that these comparisons deal only with the averages of the group and do not take into consideration plant to plant variation.

A variance analysis when applied to the data collected during each ten-day period indicated that not only was the between spray treatment variation following spraying highly significant but the variation between groups before spraying was also above the 0.01 level of probability. This was true in both runs. Since the plants were growing under similar conditions and the plants in the treatment groups had been selected at random, this before spraying variation was not anticipated.

There is evidence that, under the same treatment, the plants' transpiration rates after spraying were proportional to their rates before spraying as shown by a linear regression analysis. Therefore, by an analysis of covariance it was possible to adjust the transpiration rate after spraying to the rate of transpiration before spraying. Table 2 gives the results of this covariance analysis.

After adjusting the transpiration rates it was found that in Run Number 1 the sprayed plants, taken as a group, showed a lower rate of water loss than the control plants. In Run Number 2 when this comparison was made, this difference was found to be nonsignificant. This lack of uniformity between the two runs may be explained, in part, by the increased transpiration rates occuring during Run Number 2 (Table 1). Miller (1938) states that experiments conducted under conditions of relatively high temperatures may give less striking results from spraying compared with experiments conducted where transpiration rates are lower.

During Run Number 1, the organic sprays Zerlate, Fermate, and Deenate were not significantly different from Bordeaux mixture in their effects on transpiration, although the difference approached significance ($P = 0.08$). For Run Number 2, this same comparison produced a significant F ratio at the 0.005 level; hence it appears that after adjustment for the water loss characteristics of the plants before spraying, the Bordeaux mixture probably causes a greater water loss than do the organic sprays.

Table 2. Analysis of variance and covariance when applied to rates of transpiration for strawberry foliage.

Sources of variation	D/F	Sy^2 *	Errors of estimate			
			D/F	M. S.	F	P
Run Number 1 (April 26 to May 17, 1946)						
Total	399	33,792,448				
Between days	9	28,058,707				
Between sprays	4	460,814	4	157,661	14.10	<0.001
Control vs. all sprays	---	----------	1	256,558	22.95	<0.001
Bordeaux mixture vs. organic sprays	---	----------	1	37,486	3.35	0.080
Remainder	---	----------	2	168,300	15.05	<0.001
Day x spray interaction	36	309,501	36	8,426	0.75	>0.500
Between plants	350	4,963,426	349	11,181	-----	-------
Run Number 2 (May 17 to June 6, 1946)						
Total	389	23,325,883				
Between days	9	12,494,788				
Between sprays	4	1,719,567	4	219,848	12.43	<0.001
Control vs. all sprays	---	----------	1	37,558	2.12	>0.100
Bordeaux mixture vs. organic sprays	---	----------	1	210,840	11.92	<0.001
Remainder	---	----------	2	315,497	17.84	<0.001
Day x spray interaction	36	243,407	36	8,187	0.46	>0.500
Between plants	340	8,868,121	339	17,685	-----	-------

* Sums of squares of transpiration rates after spraying.

Differences appeared to exist among the effects of the organic sprays, but the experiment was not designed to test them legitimately. For example, after the two individual comparisons, control versus all sprays and Bordeaux mixture versus organic sprays, had been made there remained a significant amount of variation associated with the two remaining degrees of freedom. It appears that the major difference of importance lies in the contrast between the effect of Deenate W-50 and that of the other two organic sprays.

The spray comparisons stayed rather consistent from day to day as shown by the lack of a day x spray interaction. The usefulness of any conclusion drawn from this comparison, however, depends on the representativeness of the days on which the tests were made.

The technique of applying a covariance analysis rather than only a variance analysis is further justified by the fact that the plant to plant variance was reduced by 21 percent for Run Number 1 and 32 percent for Run Number 2.

Apple Tree Experiments

A discussion of a transpiration experiment in which the Heinicke and Hoffman method of measurement is used must admit the abnormal conditions under which the foliage is placed when enclosed in the leaf envelopes. It was found in the experiments covered by this paper that the temperatures inside the leaf containers varied between two and nine degrees Fahrenheit above that of the outside air. The larger differences between these two temperatures were associated with conditions of bright sun and lack of air movement outside the envelopes.

Part I. This portion of the experiments was designed in such a manner as to test the effect of successive spray applications when applied to different groups of trees. This design did not allow for replication of the individual spray applications when applied to more than one group, nor did it take into account the variation in the transpiration characteristics inherent in individual trees. The type of measurement used was such that to test the effect of successive sprays on a sufficient number of groups to make an analysis of variance practical would have involved more time than was available.

However, the results of these experiments do indicate that the differences occurring between trees, even though they are of the same age and variety, are greater than the effects of the sprays applied. For example, the Bordeaux mixture sprayed tree in Group B after five spray applications had an average transpiration rate less than the other four trees of that group; whereas the tree sprayed with this material had the highest transpiration rate of those in Group C, having received the same number of spray applications.

An analysis of variance when applied to the transpiration rate of the trees following each spray application indicated that the differences occurring between spray treatments were non-significant. Each of such analyses covered only 15 observations or a total of 14 degrees of freedom, thereby limiting significant differences to relatively large amounts. Also such analyses did not differentiate between the differences occurring between sprays and those occurring between trees, due to the design of the experiment.

There is some indication from the results obtained that there may be a day x spray interaction of plants in the field. During the tests on Group B, having received five spray applications, the Zerlate sprayed tree the day of spraying had a transpiration rate of 238.2 grams of water lost per square meter of leaf area per hour. The Deenate W-50 sprayed tree the same day had a rate of 162.5. Two days later the Zerlate sprayed tree had a rate of 131.3 as compared with 198.5 for the Deenate W-50 sprayed tree. On both days the weather was bright, sunny, and still, although on the first day the temperature was 90 degrees Fahrenheit and the relative humidity 58 percent whereas on the second day the temperature was 81 and the relative humidity 48. It should be remembered that these data are merely an example and not the result of any variance analysis.

Part II. By spraying individual branches on the same tree with the various materials under test, it was possible to eliminate the tree to tree variation found in Part I. An analysis of variance when applied to the transpiration rates, before spraying, of the branches used on the three trees in Part II indicated that the branch to branch variation on the same tree was nonsignificant. Table 3 presents the average transpiration rates of branches as influenced by single applications of the four materials tested. These averages cover the four-day period following a single spray application on each tree tested.

Table 3. Average transpiration rates, in grams of water lost per square meter of leaf area per hour, for individual branches of the three trees used in Part II of the apple experiments.

Treatment group	Tree 15 July 27-30, 1946	Tree 21 Aug. 7-10, 1946	Tree 14 Aug. 18-21, 1946	Averages of the 12 days tested
Fermate	167.0	95.5	242.8	168.4
Zerlate	131.3	97.0	268.3	165.5
Deenate W-50	134.8	87.5	182.8	135.0
Bordeaux mixture	167.0	131.8	139.8	146.2
Control	131.0	78.3	197.5	135.6

Table 4. Analysis of variance when applied to rates of transpiration for individual branches of the three trees used in Part II of the apple experiments.

Sources of variation	D/F	Sums of squares	M.S.	F	P
Total	59	225,349			
Between days	9	46,461			
Between trees	2	118,707			
Between sprays	4	13,409	3352	3.01	0.025
Control vs. all sprays	1	3,176	3176	2.85	0.100
Bordeaux mixture vs. organic sprays	1	925	925	0.83	>0.250
Remainder	2	9,308	4654	4.18	0.024
Between branches	42	46,772	1114	----	-----

An analysis of variance of the data from which these averages were determined did show that the differences between the treatments were highly significant. Individual comparisons between spray groups indicate that these differences do not lie either within the "control versus all sprays" or within the "Bordeaux mixture versus organic sprays" comparisons, therefore they must be associated in the remaining two degrees of freedom (Table 4).

The design of the experiment was not such as to permit a probability statment of a Deenate W-50 versus Fermate and Zerlate comparison. Nevertheless, it appears likely that the Deenate W-50 did effect the rates of transpiration differently than did the Fermate and Zerlate. Of the total sum of squares of 13,409 for the factor "Between sprays" the comparison Deenate W-50 versus Fermate and Zerlate accounted for 8171. If an F test were legitimate here F would be at the 0.01 level. The belief that Deenate W-50 affects transpiration differently than Fermate and Zerlate is strengthened by the fact that the same general results were obtained during the two runs on strawberry plants in the greenhouse.

Part III. The results found in this portion of the experiments are presented in graphic form in Fig. 18. Since these tests covered only two 24-hour periods and were conducted on only one tree they serve only to indicate that transpiration tests must be made at more than one period of the day to give a full account of the effects of spray materials on total transpiration.

The wide hour to hour fluctuation of the transpiration rates

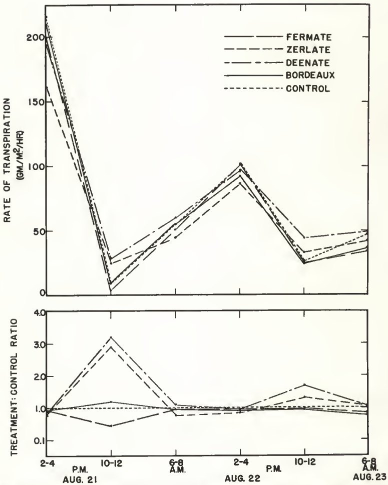

Fig. 18. The influence of the spray materials tested on the hourly rate of transpiration from apple foliage (Aug. 21, 22, and 23, 1946).

overshadowed the effects of the spray materials. When the rate of transpiration of the control branch was taken as 1.00 the relative effects of the spray materials, at the three selected periods of the day, could be compared. This comparison indicated that, following three spray applications, the transpiration rates of the branches were not constant in their relation to one another throughout the day. Deenate W-50, which in the preceeding experiments tended to be associated with lowered transpiration rates, appeared to increase transpiration at the 10:00 to 12:00 p.m. period.

The relative amount of the nighttime transpiration of Deenate W-50 sprayed foliage amounted to as much as 3.2 times that of the foliage on the control branch. The absolute amount of this increase is slight due to the reduced transpiration at night but it may, nevertheless, in part balance the daytime effects of the spray.

SUMMARY

1. There is an abundance of literature pertaining to the effect of Bordeaux mixture on the rate of transpiration. Little is known of the effects of the organic insecticide, DDT, and the newer organic fungicides on plant activity.

2. During the spring and summer, 1946, a number of experiments were conducted to determine the effects of Deenate W-50, a commercial DDT material, and Fermate and Zerlate, two commercial organic fungicides, on water loss and to compare their effects with that of 4-8-100 Bordeaux mixture.

3. These experiments were conducted on Blakemore strawberry plants grown in the greenhouse and Winesap apple trees grown in the field.

4. The weighing method of determining transpiration was used with the strawberry plants. The Freeman method as modified by Heinicke and Hoffman was adapted to measure the water loss from the foliage of the apple trees.

5. It was found highly desirable to ascertain leaf areas at frequent intervals throughout the course of the weighing experiments. This area may be estimated by obtaining leaf length x width measurements and later correlating the products of these measurements to measured leaf areas.

6. A linear regression analysis of the strawberry data indicated that a plant's transpiration rate after spraying is proportional to its rate before spraying.

7. After adjusting the transpiration rates after spraying for the rate of transpiration before spraying, it was found that under conditions of reduced transpiration the sprayed plants, as a group, had a lower rate of water loss than did the unsprayed control plants.

8. It appears that Bordeaux mixture caused a greater daily water loss than did the organic materials.

9. It also appears that Deenate W-50 causes a reduced transpiration rate from strawberry foliage when compared with Zerlate and Fermate but, due to the design of the experiment, no probability figure can be given.

10. The results of the apple tree experiments indicate that

the differences occurring between trees, even though they are of the same age and variety, are greater than the effects of the sprays applied. However, the unsprayed branch to branch variation on the same tree is nonsignificant.

11. It was found that under the field conditions encountered during the apple tree experiments there was no significant difference between the sprayed and unsprayed foliage or between the effects of the organic materials when compared with that of Bordeaux mixture.

12. Although the design of the experiment does not permit a probability statement, it appears likely that Deenate W-50 reduces transpiration from apple foliage when compared to Fermate and Zerlate. This agrees with the data obtained from the strawberry experiments.

13. Based on a limited test, it appears that the effects of the spray materials tested on the transpiration from apple foliage are not consistent throughout the light and dark periods of the 24-hour day.

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