PENETRATION, DISTRIBUTION, AND EFFECT OF PETROLEUM OILS IN APPLE[1]

By PAUL A. YOUNG

Assistant botanist and bacteriologist, Montana Agricultural Experiment Station[2]

INTRODUCTION

The objectives of the project on oil sprays of apple were to determine: (1) The concentrations of oils that apple leaves tolerate without showing necrotic symptoms, (2) the symptoms caused by petroleum oils in tissues, (3) the rates of oil penetration into leaves, (4) the distribution of petroleum oils in apple tissues, and (5) a rapid, easy, accurate method for predicting the probable injury that an oil spray will cause in apple leaves.

In a previous paper Young and Morris (*18*)[3] described the concentrations of oils in emulsion sprays that were tolerated by apple leaves, and the injuries that were caused by sprays containing 2 to 16 percent of oils. The experiments with emulsions fulfilled only the first objective of the project, because emulsions containing 1 and 2 percent of oils rarely caused symptoms in apple leaves, and emulsions containing 1 to 16 percent of oils were inadequate in determining the penetration, distribution, effect, and toxicity of each oil. Hence, undiluted oils were tested on apple leaves to fulfill the last four objectives of the project. Furthermore, sprays with undiluted-oil fogs have important advantages as commercial insecticides. The present paper describes the experiments with dissimilar undiluted oils.

REVIEW OF LITERATURE

Young and Morris (*18*) described the effects of sprays with oil emulsions on apple. They used 19 of the 29 oils described in figure 1 of the present paper and reviewed literature on the relation of viscosity to injury by oil.

Herbert (*9*) described the commercial advantages of spraying fogs of nearly pure oils on deciduous trees.

Young[4][5][6] (*15*, *16*, *17*) described symptoms caused by oils, the parts of tissues containing oils, and freezing phenomena in spray-oil emulsions. He explained theoretically how oils enter protoplasm and showed that *Rhizopus nigricans* Ehr. can be used to indicate the probable effects of oils on apple leaves.

Groves, as reported by Kendall (*11*), found that oil replaced the air in the intercellular spaces of leaves, causing translucence; the oil remained indefinitely in the positions first occupied. English (*2*) wrote

[1] Received for publication Feb. 17, 1934; issued October 1934. Contribution from Montana State College, Agricultural Experiment Station, Paper 42, Journal Series.

[2] The writer wishes to thank Professors H. E. Morris, D. B. Swingle, F. B. Cotner, and J. R. Green, of the Montana Agricultural Experiment Station and College of Agriculture, for criticizing this manuscript.

[3] Reference is made by number (italic) to Literature Cited, p. —.

[4] YOUNG, P. A. SPRAY OIL PENETRATION OF APPLE LEAVES, LIMBS AND FRUIT. Abstract of Papers, Amer. Soc. Plant Physiol., Des Moines, Iowa, pp. 11–12. 1929. [Mimeographed.]

[5] ——— OIL-MASS THEORY OF PETROLEUM-OIL PENETRATION INTO PROTOPLASM. Abstract of Papers, Bot. Soc. Amer., Boston, Mass., pp. 11–12. 1933. [Mimeographed.]

[6] ——— FREEZING PHENOMENA IN CRESOAP EMULSIONS OF PETROLEUM OILS. Abstract of Papers, Amer. Soc. Plant Physiol., Boston, Mass., pp. 14–15. 1933. [Mimeographed.]

that pure 7- to 9-percent sulphonatable oils blackened tissues within 2 days, while pure 0- to 3-percent sulphonatable oils slowly caused chlorosis and defoliation. Kelley (*10*) stated that oil emulsions entered the intercellular spaces of leaves. Ginsburg (*3*, *4*) determined that oils penetrate apple leaves at rates inversely proportional to the viscosities and directly proportional to the concentrations of the oils.

Bartholomew (*1*) reported that oil entered through both sides of citrus leaves and passed between and into the cells. Knight, Chamberlin, and Samuels (*12*) illustrated oils in and between the cells of orange leaves and stated that oils passed into the phloem, medullary rays, and pith of stems. Proescher (*13*) used Oil Red O, and Haynes (*8*) used Sudan IV for staining oils.

Woglum, LaFollette, Landon, and Lewis (*14*) observed that oil sprays on citrus trees were associated with an increase in dead wood, fruit dropping, culled fruit, and navel water rot.

Harvey (*7*) described the pneumatic and hydrostatic systems in apple, as studied by injected aqueous solutions of dyes, and reviewed literature on this subject.

Green (*6*) found evidence that filtration, sulphonation, and alcoholic extraction removed some of the toxic materials in oils.

MATERIALS AND METHODS

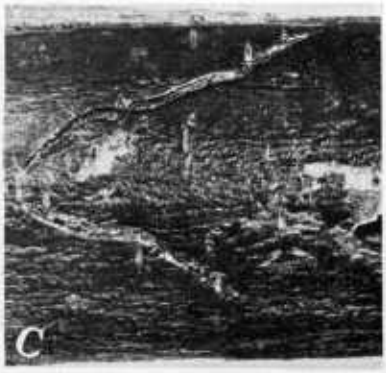
The petroleum oils listed in figure 1 were tested on Hibernal apple in an orchard on the farm of the Montana Agricultural Experiment Station at Bozeman, Mont., during 1926 to 1933. Before using, some samples of oils 3, 13, and 24 were saturated with Oil Red O to make them easily discernible in tissues. Indophenol Blue was used in one sample of oil 24. The oils were painted in undiluted form on apple leaves to determine promptly the effect of each oil. Undiluted oils were used, except where otherwise stated.

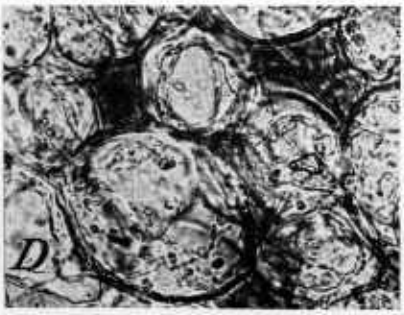
The distribution of oils in apple tissues was determined by sectioning oil-treated leaves and their twigs 1 to 15 cm from the leaves. The tissues were sectioned 1 to 488 (usually 8 to 80) days after the oils were placed on the leaves. Oils were placed on apple fruits, and sections were made later to determine the penetration and distribution of oils in the fruits. The sections were studied with and without staining. Tissues separately containing 10 of the oils were studied on 600 slides. Comparable leaves, stems, and fruits to which no oils had been applied were sectioned, stained, and studied for comparison with the oiled tissues.

The following methods were used in staining oils:

Sections were cut from freshly collected leaves and stems, and were stored in water for a few minutes. They were then (1) placed in equal parts of pure acetone and aqueous solution of 70-percent ethyl alcohol for a few minutes to remove chlorophyll and prepare them for staining, (2) stained for 1 to 3 minutes in a saturated solution of Sudan IV in equal parts of acetone and 70-percent ethyl alcohol, (3) destained for 1 to 3 minutes in the alcohol-acetone mixture, (4) washed for a few minutes in water, (5) counterstained for 1 to 3 minutes in a solution of 10-percent Unna's alkaline methylene blue, (6) washed in water for a few minutes, (7) mounted on slides in pure glycerine, and (8) sealed on the slides with clear Duco varnish.

Tissues were preserved with water and were cut soon after collecting, or were stored in a 60-percent solution of pyridine during 1 to 60





A, Angular mottling (white) representing oil 21 in polyhedrons of mesophyll in an apple leaf. This leaf was excised and dried 8 days after oiling and was photographed 348 days after oiliug, showing that the vein-bounded polyhedrons of mesophyll held the oil; 384 days later, only one oily-translucent polyhedron remained in this leaf, showing that most of the oil had disappeared from the other polyhedrons that were occupied by oil when photographed. Irregular brown lines (photographed black) are shown. Mesophyll enclosed by these lines resembled that outside them. ×1. *B*, Like *A*, but photographed with a brown filter so that the brown lines and spots photographed white. Parts of lamina occupied by oil 21 photographed gray, while the normal, nonoily parts of the lamina photographed black. This leaf was photographed 8 days after oiling. In contrast, it showed no oily translucence 732 days after oiling. ×80/107. *C*, Oil-canker line separating living and dead bark in an apple limb 219 days after nearly 10 cc of oil 24 saturated with Oil Red O had been injected into this limb through a glass tube. The canker line formed nearly 180 days after the oil injection, and the leaves on this limb died 275 days after this oil injection. ×2. *D*, Oil (black) between the receptacle-cortex cells of an apple fruit 2 days after oil 24 saturated with Oil Red O had twice filled the calyx basin of this apple in the laboratory. ×149.

days. They were sectioned freehand or with a microtome. Most of the sections were cut from living tissues, but some pieces of leaves were frozen with solid carbon dioxide or in water. Sections were then (1) washed in running water, (2) immersed in a 60-percent solution of pyridine for a few minutes to remove chlorophyll and prepare them for staining, (3) stained for a few hours in Oil Red O dissolved in a 70-percent solution of pyridine, (4) destained for a few minutes in 60-percent pyridine, (5) washed in running water for a few minutes, (6) counterstained with light green SF, (7) mounted on slides in pure glycerine, and (8) sealed on the slides with clear Duco varnish.

Oils 3, 13, and 24 were injected into apple limbs and trunks to study the distribution and effects of large amounts of the oils. Holes 1 to 2 cm deep and 3 or 7 mm in diameter were bored into 90 apple limbs and trunks. Glass tubes 8 to 25 cm long and 3 or 7 (mostly 7) mm in diameter were set in the holes and sealed with grafting wax (pl. 2, *H*). The larger tubes had a capacity of 0.36 cc per 1 cm while the smaller tubes had a capacity of 0.16 cc per 1 cm. Additional quantities of oils were placed in the tubes 6 to 9 times during 2 weeks after starting. Oils containing 0.2 percent Oil Red O were used in most of the tubes to make easy the detection of the oils in the trees, but unstained oils were used in many tubes.

L-shaped iron tubes with inner diameters of 6 mm, outer diameters of 11 mm, and sides 8 to 10 cm long were set in holes 2 to 3 cm deep in 22 apple limbs and trunks. Reservoir holes 3 to 6 mm deep and 3 mm in diameter were bored in the bottom of each hole. The tubes were filled with measured amounts (5 to 9 cc) of oil 24 saturated with Oil Red O, and the free ends were covered with glass vials.

EXPERIMENTAL RESULTS

OIL PENETRATION INTO LEAVES

The oils shown in figure 1 entered living leaves of apple, *Pyrus malus* L., and caused translucence by filling many of the intercellular spaces. Hypophyllous applications of oils to normal leaves at 15 to 25° C. revealed the following rates of penetration causing immediate translucence: 2 to 60 seconds for the oils with viscosities of 50 to 108 seconds, and 5 to 30 minutes for oil 8 with a viscosity of 410 seconds. Thus, oils with a low viscosity penetrated more rapidly than those with a high viscosity.

During penetration, the oils inside the leaves commonly spread beyond the margins of the superficial drops of oils. The translucence of the oily spots varied much apparently because different amounts of oils occupied the intercellular spaces in the different vein-bounded polyhedrons of mesophyll. These oily, vein-bounded spots in the leaves were distributed discontinuously, or occupied large regions, many of which surrounded nonoily parts of mesophyll (pl. 1, *B*). This caused the oily sign, angular mottling (pl. 1, *A*). Angular mottling was caused by undiluted oils, and by calcium-caseinate emulsions of 2 to 16 percent of oils that probably penetrated the leaves as free oils. The amount of oil penetrating determined the size and translucence of the angular mottled spots.

Oily spots in living green leaves and in dried specimens of such leaves were translucent and yellowish green by transmitted light, and were blackish green by reflected light in contrast to the nonoily

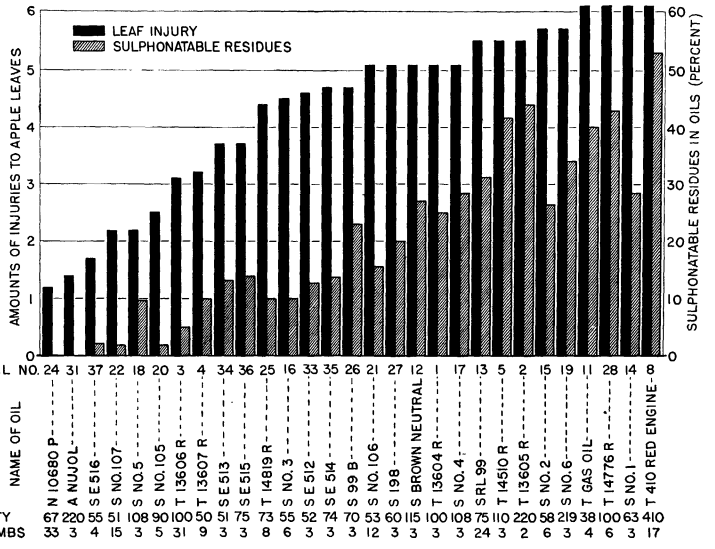


FIGURE 1.—Injuries to apple leaves caused by oils as compared with the sulphonatable residues of the oils. Amounts of oil injuries to leaves shown by solid bars are indicated by numbers that signify as follows: 1, No necrotic symptoms appeared within 22 days after the leaves were oiled, and the bark on the attached twigs was alive from 85 to 145 days after their leaves were oiled; some of the oiled leaves showed no necrotic symptoms within 30 to 60 days. 2, Some mild, necrotic symptoms appeared within 8 to 22 days, and the bark on the twigs was alive from 85 to 145 days after the attached leaves were oiled. 3 like 2, except the leaf symptoms were more abundant and severe. 4, Some severe necrotic symptoms appeared within 8 days, and part of the bark on the attached twigs died within 100 to 145 days. 5, Severe and abundant necrotic symptoms appeared within 8 days, and much of the bark on the attached twigs died within 85 to 145 days. 6, Many oily parts of leaves died within 1 day, or part of the bark on the attached twigs died within 39 days. The shaded bars show the percentage of sulphonatable residue in each oil as determined by the makers of the oils, except oils 5, 13, 15, 16, and 21, the sulphonatable residues of which were determined by Green (*5*) who also used these spray oil numbers. Below are given serial numbers of the petroleum oils, viscosity of each oil is seconds (Saybolt universal viscosimeter) as determined by the oil companies, number of limbs on the leaves of which sufficient oils were placed to make 20 to 90 percent of the laminae translucent, and names or company numbers of the oils, with symbols indicating the names of the oil companies, which signify as follows: N, is L. Sonneborn Sons, Inc., New York; A, is Stanco, Inc.; I, is Standard Oil Co., of California; S, is Shell Oil Co.

parts of the same leaves. Thus, light was absorbed less in refraction and more in reflection by the oily spots than by the nonoily parts.

The appearance of the oily spots in leaves became nearly stable within 2 to 60 minutes after oil penetration started. Thereafter, the sizes, shapes, colors, and translucence of the oily spots in the

EXPLANATORY LEGEND FOR PLATE 2

The oils appear black in these photographs.

A.—Section of an apple limb several centimeters below a glass tube through which nearly 5 cc of oil 24 saturated with Oil Red O was injected 219 days previously. The large, red-oily sector shows where much oil entered the xylem. ×1.

B.—Oil in tracheae of an apple-leaf petiole 32 days after oil 24 was placed on its lamina. ×293.

C.—Oil between the cells of an apple fruit 11 days after oil 8 was painted on the epidermis. ×123.

D.—Raised, yellow blisters surrounding brown depressions around the lenticels in apple bark 213 days after oil 3 saturated with Oil Red O was injected into this limb through a glass tube. The limb then bore normal leaves. ×9/4.

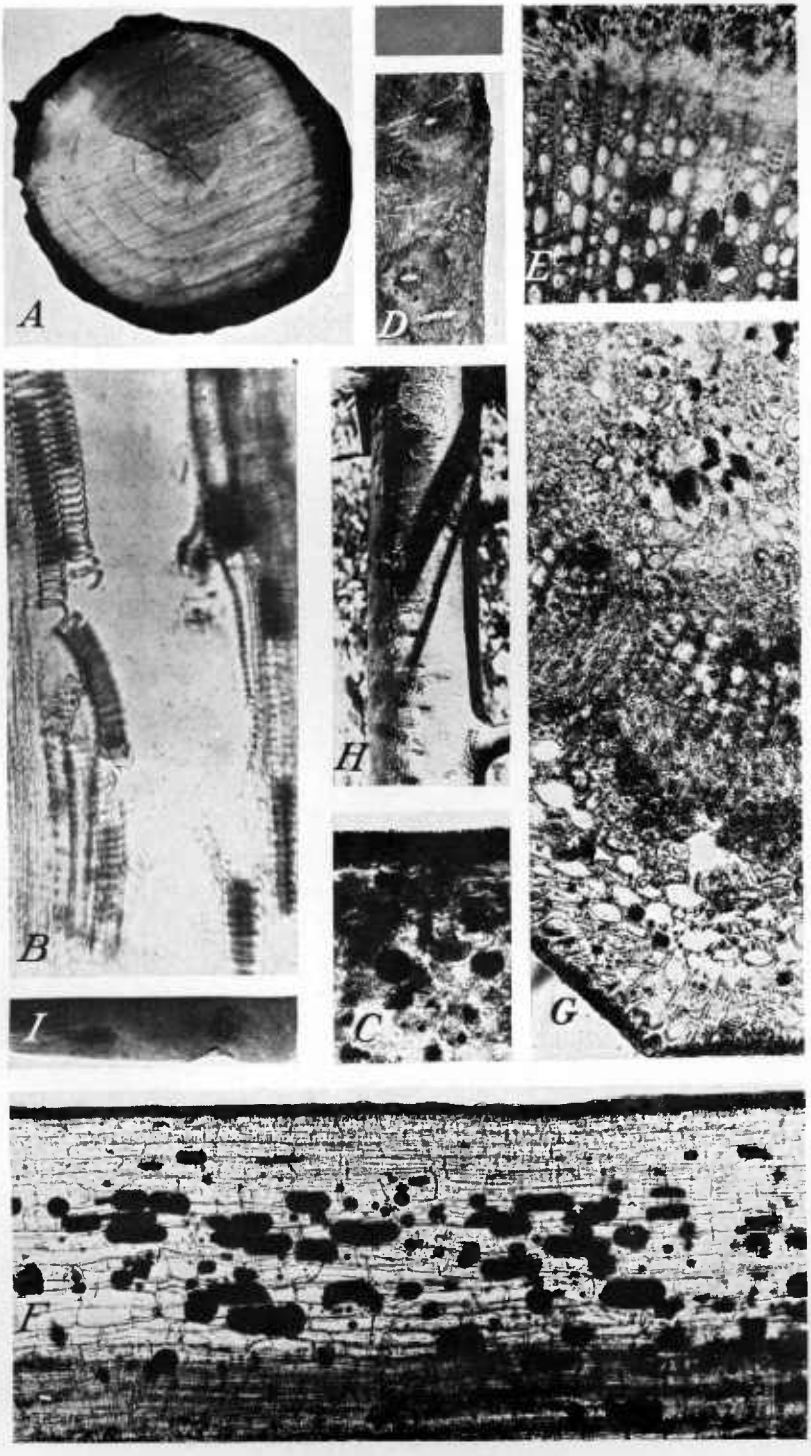
E.—Oil inside tracheae and phloem of a 2-year-old apple twig 109 days after the attached leaves were sprayed with 16-percent oil 24 emulsified with calcium caseinate. The oil had passed from the leaves into this twig. ×93.

F.—Oil inside and between parenchyma cells of an apple-leaf petiole 32 days after oil 24 was placed on the attached lamina. ×85.

G.—Oil in the tracheae and in the epiphyllous and hypophyllous parenchyma cells of the midrib of an apple leaf 30 days after oil 8 was applied to its lamina. ×137.

H.—Raised, interlenticel, yellow and gray blisters near the ink label on the bark of this apple trunk into which nearly 180 cc of oil 24 saturated with Oil Red O entered through four glass tubes (one shown at the top) 220 days previously. The oil was injected on October 25 to November 3, 1930. A field of *Cytospora* pyenidia (at top) appeared in bark killed by oil. ×1/4.

I.—Yellow blisters in the trunk bark of an apple tree 220 days after oil 24 saturated with Oil Red O was injected into the trunk 34 cm above them. ×9/7.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

living leaves changed very little. This indicated that only negligible amounts of oils evaporated from the interiors of living leaves that had been treated with oils having viscosities of 50 to 410 seconds.

This conclusion that oils disappear only slowly from the interiors of leaves was supported by observations on many oil-mottled leaves that were dried and stored in the herbarium. During the first 1 to 3 years, the oily spots in these dried leaves commonly retained essentially the appearances that they had while the leaves were alive. The following data give the periods of time during which parts of many of the dried leaves remained prominently oily translucent by transmitted light, and black by reflected light: Oil 3, 5.5 years; oils 8, 13, and 21, 4.3 years (pl. 1, *A*, *B*); oil 24, 3.3 years; and oils 1, 5, 12, 26, and 36, 2.4 years. Those of the herbarium specimens of leaves that contained the most viscous oils showed the most numerous, large, and prominently oily spots.

Hypophyllous strips were taken from mature and juvenile apple leaves on several dates and were placed immediately in distilled water. Prompt microscopical examinations showed that the stomatal orifices had dimensions of 10μ to 30μ by 2μ to 7μ, while the surrounding pairs of guard cells had dimensions of 27μ to 38μ by 24μ to 34μ. Thus, the stomatal orifices were large enough to permit rapid flow of oils through them.

OILS IN LEAVES AND TWIGS

Ten oils (table 1) penetrated and spread similarly, so their range of 50 to 108 seconds in viscosities made no apparent difference. These oils occupied the tissues described in table 1 and illustrated in plates 1, *D*, and 2, *B*, *E*, *F*, *G*.

Similar distribution of most of these oils was observed after the leaves were sprayed with emulsions containing 2, 4, 8, and 16 percent of oils. The different concentrations of oil placed on the leaves affected only the amounts of oil in the tissues, as more oil entered the tissues from the undiluted oil than from the emulsions. The globules and distorted masses of oils seen in apple tissues usually were 8μ to 70μ in diameter. No oils were found in any of the unoiled tissues studied.

TABLE 1.—*Locations of petroleum oils inside and between cells of apple leaves and their attached petioles and limbs after the undiluted oils had been painted on the leaves* [1]

General location of oils	Oils in leaf laminae				Oils in attached petioles		Oils that passed from oiled leaves into attached limbs 1 to 4 years old					
	Spongy parenchyma	Palisade parenchyma	Vein tracheae	Vein parenchyma	Tracheae	Parenchyma	Pith	Tracheae	Medullary rays	Phloem	Cortical parenchyma	Lenticel parenchyma
Inside cells	O	O	A	CP	A	CP	CP	A	CP	BC	BC	O
Between cells	A	CP	O	CP	O	CP	A	O	O	CP	A	CP

[1] Relative amounts of oils in and between cells are indicated by letters that signify as follows: O, no oil seen; BC, oils seen in or between some cells; CP, oils commonly present; A, oils abundant. The distribution of the following oils in these tissues were studied: Oils 3, 5, 13, 18, 20, 21, 22, 24, 25, and 28 (fig. 1).

Typical Examples of Oils in Apple Tissues

An ammonia-casein emulsion of 8-percent oil 21 was sprayed on apple leaves on June 6, 1930. Sections of the leaves, cut 34 days later, showed oil inside the vein tracheae and some vein parenchyma cells, and between the phloem, spongy, and palisade parenchyma cells of the veins. Sections of the attached 1930 stem showed oil between the parenchyma cells near the bast, between collenchyma, lenticel, and pith cells; and inside pith cells, tracheae, tracheids, and some phloem cells.

A calcium caseinate emulsion of 16-percent oil 24 was sprayed on apple leaves on seven limbs on July 12, 1929. This oil was found 488 days later in the attached stems inside the tracheae, tracheids, pith, parenchyma, and medullary ray cells; and between pith, phloem, and cortical parenchyma cells. Some oil was found in the 1927 wood. Later sections showed some oil in 1930 wood, showing that the oil was moved from the old wood into the new wood.

An apple leaf was sectioned 7 days after oil 24 was placed on it. The oil was seen in the substomatal cavities, between chlorenchyma cells, and inside vein tracheae and vein parenchyma cells. Sections of the attached twig revealed oil in tracheae, and in and between cortical parenchyma and pith cells.

Apple twigs were sectioned 47 to 77 days after oil 3 was placed on the attached leaves. The oil was seen inside wood tracheae formed during the last 4 years; inside pith, cortical parenchyma, and old phloem cells; and between collenchyma and cortical parenchyma cells.

Oil 8, placed on apple leaves, was later found in the following tissues: Inside the tracheae, and inside and between the parenchyma cells of leaf veins and a midrib (pl. 2, *G*); between the spongy parenchyma cells of leaves; and inside the tracheae and some parenchyma cells of attached twigs. Like the other oils, this very viscous oil also was distributed in the tissues.

Sections showed that the thick-walled cylinders of cells were incomplete on most leaf veins, and were apparently inadequate barriers to the movement of large amounts of oils from the intercellular spaces of leaves into their tracheae. Large amounts of oils commonly passed from leaves into their petioles and twigs.

Distribution and Effect of Large Amounts of Oils in Limbs

The effects of large quantities of oils in tissues were studied by separately injecting oils 3, 13, and 24 through glass tubes into apple limbs and trunks during October 1930. Five to fifty (usually 10 to 25) cubic centimeters of oil passed from each tube into its limb or trunk. The bark usually became oily within 10 to 15 cm from the glass tubes. Oil 13 killed the injected limbs within a few weeks, while oils 3 and 24 killed the injected limbs within 6 to 10 months. Similar results followed the use of stained and unstained oils. Check holes in limbs did not seriously injure the limbs.

On June 5, 1930, oil 24 with the Oil Red O stain was placed directly in drill holes in eight apple limbs, without using glass or iron tubes; 8 to 10 cubic centimeters of oil passed into each limb.

Ten limbs and three trunks were sawed 139 days after oiling in the June series, and 9 to 12 months after oiling in the October series. When oil was not visible immediately on cut surfaces, they were made

smoother and observed 15 minutes later to permit oil present in the tracheae to flow out and become apparent. In these limbs and trunks, red-stained oils were found in the wood 5 to 88 (average of 48) cm below the oil-injection holes (pl. 2, *A*). Oil was found 51 cm above one oil hole. The oils in the limbs occurred mainly in the tracheae, especially in the tracheae and sides of annual rings vertically connected with the oil-injection holes.

Oil 3 was injected through a glass tube into a limb. Sections 10 cm beyond the glass tube were cut 8 days later. They showed oil in tracheae, in and betweeen cortical parenchyma cells, and between collenchyma cells.

Compressed air was used to facilitate oil penetration in other tests. Six of the L-shaped iron tubes were set in holes in apple limbs on June 3 and 20, 1932. Oil 24 saturated with Oil Red O was placed in each tube. Air pressures of 10 to 25 pounds per square inch were applied to each tube, and the compressed air forced 1 to 3 cc of oil into each limb within 7 to 60 minutes. Thus, the compressed air greatly accelerated the penetration of the oil and was the best method of injecting oil into apple limbs. The limbs were sawed 10 to 267 minutes later; red oil was revealed in the tracheae 9 to 49 cm above and below the iron tubes. The oil was conspicuous only in the annual rings vertically connected with the oil reservoirs of the iron tubes. The oil occurred mainly in the inner tracheae of 2 annual rings in 1 limb, and mainly in the central tracheae of 2 annual rings in 2 other limbs, thus showing the tracheae functioning at this time.

OIL PENETRATION INTO APPLE FRUITS

Previous experiments showed that some sprays of oil emulsions caused necrotic spots in apple fruits. Evidently oils entered these apples, so experiments were conducted to test the penetration of apple fruits by oils.

Oils 3, 8, 13, and 24 were placed separately as drops on many apples in the orchard. They soon made oily spots that, by reflected light, were darker colored than adjacent unoiled regions. Oils 3 and 24 caused no necrotic symptoms, while oils 8 and 13 caused blackening and browning of cells in and near the oiled lenticels.

Oils 3 and 24, saturated with Oil Red O, were placed separately as drops on 50 green apples in the orchard. These oils entered through the lenticels and caused oily spots in the apples within 5 to 60 minutes. Strips of oiled epidermis from these apples showed that the lenticels were oily red while the remainder of the epidermis was hyaline. Parenchyma cells and some tracheae within 1 mm of the epidermis were red with oil. Most of the oil was between the parenchyma cells (pl. 1, *D* and 2, *C*).

The stem cavities and calyx basins of 46 ripe Hibernal, Wealthy, and Winesap apples were filled 2 to 5 times with oils 3 and 24 (with and without Oil Red O), used separately in four series of experiments in the laboratory. Each apple absorbed traces to 5 cc of oil within 1 to 4 weeks. Open calyx tubes and orifices around the stems in some apples permitted oils to flow rapidly into the core cavities. No difference was observed in the behavior of the stained and unstained oils. Sections of the oiled apples were examined within 5 to 30 (mostly 7 to 10) days after oiling. Each oiled apple showed a

cone-shaped, very oily layer of tissues 3 to 8 mm thick adjacent to, and below, the oiled stem cavities and calyx basins. Such layers had abundant oil in and between the parenchyma cells (pl. 1, *D*). Some core cavities contained much oil. Some vessels radiating from oily cores were pink with red-stained oils, and oils were seen in some tracheae.

SYMPTOMS CAUSED BY UNDILUTED OILS IN APPLE LEAVES, BARK, AND BUDS

Brown and Dry Green Spots Preceding Defoliation

Oily translucent mesophyll (pl. 1, *B*) commonly died, became dry, and turned dark brown or light green. Dark brown or purple irregular lines or concentric-arc lines 0.5 to 2 mm wide appeared mostly within 39 days and bounded many of these spots (pl. 1, *A*). Some of the purple and brown borders were bounded by nonnecrotic, oily translucent mesophyll. Angular mottling commonly occurred in other parts of the laminae (pl. 1, *B*). The unsulphonatable oil 24 required 3 to 9 weeks to cause these symptoms, while most of the other oils caused such brown spots within 0.5 to 2 weeks. Indophenol blue apparently increased the toxicity of oil 24. Leaves with 50 to 90 percent of the laminae killed by oils usually fell off their trees one to many weeks before normal defoliation. The rapidity of appearance and the abundance of the necrotic leaf spots was directly correlated with the percentages of the sulphonatable residues in the oils (fig. 1).

Epiphyllous Purple Spots

Oil 18 and the oils more than 13 percent sulphonatable (except oils 17, 28, 34, and 36) caused prominent epiphyllous purple spots. Evidently only these oils contained sufficient concentrations of the chemicals that cause this symptom. Thus, this symptom distinguished many oils that were very toxic to apple leaves.

White and Light Brown Epiphyllous Leaf Spots

Oil 11 caused irregular, epiphyllous, white to light brown, usually purple-bordered spots 0.5 to 2 cm wide within 39 days. Some of these spots were hypophyllously green and apparently alive, but most of them were necrotic and hypophyllously dark brown. The fact that only oil 11 caused these symptoms indicates that only it contained the causal chemical in sufficiently concentrated form.

Flavescence

Unlike chlorosis, flavescence describes the abnormal, nonnecrotic, light green to yellow colors in leaves that never were normally green. Flavescent, dwarfed leaves were developed by buds on several limbs girdled by oils 3 and 24 that were injected through glass tubes during the preceding October. Also, slender, rolled, dwarfed, flavescent leaves were developed by the buds of three branches, the leaves of which had been sprayed with 8- and 16-percent oil emulsions 10 and 22 months previously. Most of the flavescent leaves died within a few weeks after appearing.

Leaf Dwarfing and Drying

Leaves delayed in developing, often flavescent, and only 0.5 to 2 cm long, were produced by many buds 60 days after treatment with oils 3 and 24, and by many buds on some limbs injured 6 months previously by these oils injected through glass tubes. Leaves 0.5 to 5 cm long were produced by 60 other limbs into which oils were injected through glass tubes. These leaves were normally green when they appeared, but during the summer they developed similar symptoms of chlorosis, wilting, and drying, and all of them died 2 to 5 months earlier than the leaves on normal limbs. Most of the dried leaves remained attached to their branches during the remainder of the summer. The dying of the leaves usually accompanied the dying of the bark on their branches.

Six of the oils applied in concentrations of 16, 90, and 100 percent killed the leaves on 25 limbs without causing abscission. These leaves became brown and dry, later turned gray, and thereafter remained attached to their branches during 1 to 6 months.

Buds and Twigs Killed by Oil

The buds on 37 dormant limbs were treated separately with oils 3, 12, 15, 16, and 24. Oils 3 and 24 killed a few of the buds on each limb and caused dwarfing of the leaves formed by some of the other buds. In contrast, the more sulphonatable oils 12, 15, and 16 killed most of the treated buds.

The oils (fig. 1) applied in concentrations of 8, 16, 90, and 100 percent to apple leaves entered and killed 150 of the attached twigs within 6 months. For example, oils 3 and 24 killed the tips of such twigs within 3 to 6 months, while the more sulphonatable oil 13 killed large parts of the small limbs within 2 to 7 weeks. These data emphasize the conclusion that the toxicity of an oil is closely correlated with its sulphonatable residue.

Oil Cankers and Narrow Canker Lines in Bark

Narrow canker lines separating living and dead bark developed within 3 to 9 months in nearly half of the 150 oil-killed twigs. Many oil cankers were conspicuous during 1 to 2 months before the canker lines appeared. Two to five canker lines appeared in each of several twigs.

In May 1931 the bark looked normal surrounding the glass tubes through which oils were injected into 90 limbs in October 1930. These limbs developed normal-appearing new leaves. During June to September 1931, cankers which were 5 to 70 cm long and which girdled their limbs developed in the bark around all these glass tubes. Cracks separating living and dead bark formed in 20 of these cankers (pl. 1, *C*) while 4 of the cankers lacked distinct margins. The mature cankers were depressed, with green, gray, orange, or yellow outer surfaces. Within 1 to 8 weeks after girdling cankers developed, the bark shriveled and the leaves withered beyond the cankers.

Yellow and Gray Blisters in Bark of Limbs

Yellow and gray blisters 1 to 3 mm high and 5 to 15 mm wide formed on 17 cankers caused by oils near glass tubes (pl. 2, *D*, *H*, *I*). The groups of blisters were 2 to 15 cm wide and usually were within 5 to

20 cm from the glass tubes. The cortex in the blisters was soft and spongy, with lenticels in the depressions between the blisters. These blisters collapsed within a month, leaving yellow to gray, depressed, cracking cankers from which many parts of the paper-like epidermis separated.

Much oil had entered the bark near the glass tubes. The large amount of oils in the bark tissues presumably decreased the entrance of oxygen and the elimination of water and carbon dioxide. When excess water makes conditions in tissues similarly abnormal, many tissues react by developing intumescences and oedema, as described in the literature. This explanation logically applies to such abnormalities mainly caused by excess oil. Furthermore, by decreasing transpiration, oil might result in excess water in bark tissues and thus cause intumescences.

DISCUSSION

The sizes, shapes, and translucence of oily spots in apple leaves became nearly stable within 2 to 60 minutes after oiling and changed little during the remainder of the growing seasons. Evaporation of the oils from the interiors of living leaves in orchards apparently is negligible. Much of the oil is retained by the leaves and thus is removed from the trees by defoliation. This removal of oils, and the resistance of apple to oils help to explain why there usually is no cumulative injury by oils sprayed on apple leaves.

Low-viscosity oils penetrated apple leaves more rapidly than did high-viscosity oils. The amount of oil penetrating tissues depended on the amount of oil available and the capacity of the tissues. The oils considered here ranged in viscosities from 38 to 410 seconds and were tested in undiluted form. Difference in viscosities was not evidently responsible for different effects of the oils in the apple leaves.

Methods of purifying oils deserve more attention. Oils 21, 33, and 35 were sulphonated with liquid sulphur dioxide in contrast to the comparable oils 34 and 36 that were sulphonated with sulphuric acid. These five oils were stated as having almost identical sulphonatable residues, yet the oils sulphonated with sulphur dioxide were more injurious than those sulphonated with sulphuric acid. Probably sulphuric acid removed more of the toxic chemicals from the oils than did sulphur dioxide. Hence, a statement of the sulphonatable residue of an oil should include a statement of the method of sulphonation. However, the method of sulphonation may be unimportant in commercial sprays with emulsions of only 0.5-percent oil.

Large amounts of oils passed from oil-treated apple leaves into their petioles and stems, as shown by the sections of tissues. The oils were distributed extensively in and between the parenchyma cells, and in the tracheae of leaves, petioles, and twigs. The oils prominently revealed the extensive system of intercellular spaces in the parenchymatous tissues of apple leaves, stems, and fruits. Intercellular spaces are assumed to be physiologically valuable in the distribution of gases in tissues. The diffusion and distribution of water, carbon dioxide, and oxygen is retarded by oils that obstruct stomata and occupy tracheae and intercellular spaces. Hence, oils presumably are physically injurious when they are abundant in apple tissues.

The physical and chemical effects of the oils are contrasted in figure 1 in which the chemical or toxic injuries are represented mainly by the cross-lined bars, while the physical injuries are represented mainly by the parts of the solid bars that project above the tops of the cross-lined bars. The percentages of sulphonatable residues did not indicate accurately the toxic effects of some oils. For example, oil 18 was less toxic than its sulphonatable residue indicated. In contrast, oils 14 and 15 were more toxic than their percentages of sulphonatable residues indicated, probably because some of their chemicals were unusually poisonous. However, the oils that were more than 15 percent sulphonatable, and that caused injuries in amounts of 5 to 6 (fig. 1) were too toxic for commercial use in sprays of oil emulsions on apple leaves.

Physical and chemical injuries are best considered to be fundamentally different. For example, protoplasm dies from physical injury when it is heated or ruptured excessively, or when it lacks sufficient oxygen. In contrast, protoplasm dies from chemical injury when poisonous chemicals react with it.

Toxicity is strictly a chemical property of a substance. The toxicity (total amount of chemical effect) of a substance is modified by (1) the resistance of the affected protoplasm, (2) the oil concentration above the maximum amount tolerable, (3) the temperature of the toxic chemical, and (4) the duration of its toxic action on protoplasm. The volatility of a chemical may affect the duration of its action.

Consequently, the toxicity of an oil depends mainly on its chemically active, mostly sulphonatable parts. These parts differ in their effects on apple. The concentration of oil in a spray should not far exceed the resistance of apple protoplasm to the toxic parts of the oil.

The toxic effects of each undiluted oil depended mainly on the percentage and chemical nature of its sulphonatable residue. Quick killing characterized the primary effect of very toxic oils. Tissues tolerated small amounts of toxic oils and larger amounts of nearly nontoxic oils, but they were badly injured by very toxic oils and by large amounts of less toxic oils. Large amounts of oils passing from leaves into their twigs usually killed the twigs within 9 months.

SUMMARY

Large amounts of oils passed from oiled leaves of apple into their twigs. The oils were widely distributed in and between the parenchyma cells, and in the tracheae of leaves and their twigs. Oil was found in an apple stem 488 days after the oil was sprayed on the leaves then attached to this stem.

Oils prominently revealed the extensive system of intercellular spaces in parenchyma tissues. Large amounts of oils in these spaces are physically injurious because the oils retard the diffusion of oxygen and carbon dioxide. Oils in tracheae retard the distribution of water.

Petroleum oils with viscosities of 50 to 108 seconds hypophyllously penetrated apple leaves and made translucent spots within 2 to 60 seconds. The stomatal orifices in the leaves were 10μ to 30μ by 2μ to 7μ, which permitted the oils to flow rapidly into the leaves.

Oily spots in leaves were translucent and yellowish green by transmitted light, and were blackish green by reflected light, in contrast to the nonoily parts of the same leaves. Evidently, light was absorbed less in refraction and more in reflection by the oily spots than by the nonoily parts.

Oils injected through glass tubes into apple limbs were found 9 to 12 months later in annual rings 5 to 88 cm from the points of injection. The oils were mainly in the tracheae of annual rings vertically connected with the points of injection. Pressure injections of red-stained oils into apple limbs revealed the parts of the annual rings then used in conduction.

Drops of oils on apple fruits penetrated through the lenticels and passed between the fruit-parenchyma cells.

The main symptoms caused by oils in apple leaves were: (1) Brown and dry green spots many of which had brown or purple borders; (2) epiphyllous purple and white spots that indicated very toxic oils; (3) leaf dwarfing; (4) leaf dying and dropping.

The main symptoms caused by oils in apple limbs were: (1) Killing of buds, twigs, and bark; (2) narrow canker lines in bark, and (3) yellow and gray blisters in bark.

Toxicity is a chemical property of an oil. The toxicity of an oil to apple is modified by: (1) The resistance of the protoplasm; (2) the oil concentration above the amount tolerable; (3) the temperature; and (4) the duration of oil action on protoplasm. There was no apparent correlation between the amounts of oil injury to apple leaves and oil viscosities ranging from 38 to 410 seconds.

The toxic effects of the oils depend mainly on the percentages of their sulphonatable residues. However, white leaf spots and epiphyllous purple spots were caused only by certain oils, which showed that the chemical natures as well as the percentages of sulphonatable residues influence the symptoms of oil injuries.

Tests with undiluted oils on apple leaves have an immediately practical value. Toxic effects of oils in apple leaves were determined accurately, easily, and quickly by placing drops of each undiluted oil on the lower sides of normal, mature apple leaves. Unsulphonatable oils were used for comparison. The rapidity, severity, and abundance with which symptoms of injuries appeared showed the toxicity of each oil to apple leaves. The oils that killed large parts of leaves within a week were too injurious for use in oil sprays on apple leaves. Thus, the probable injurious effects of an oil can be tested before it is used commercially in sprays on apple leaves.

LITERATURE CITED

(1) Bartholomew, E. T.
1929. penetration of spray oil into citrus tree tissues. Calif. Citrogr. 14: 337.

(2) English, L. L.
1928. some properties of oil emulsions influencing insecticidal efficiency. Ill. Nat. Hist. Survey. Bull. 17: 235–259, illus.

(3) Ginsburg, J. M.
1930. studies on penetration of oils into plant tissues. N.J. Agr. Expt. Sta. Rept. 51: 163–167.

(4) ———
1931. penetration of petroleum oil into plant tissue. Jour. Agr. Research 43: 469–474.

(5) GREEN, J. R.
1932. CHEMICAL AND PHYSICAL PROPERTIES OF PETROLEUM SPRAY OILS. Jour. Agr. Research 44: 773–787, illus.
(6) ———
1933. EXPLAIN MEANS OF IMPROVING SPRAY OILS IN ORDER TO MINIMIZE INJURY TO GROWING PLANTS. Oil and Gas Jour. 31 (37): 8, 29, illus.
(7) HARVEY, E. M.
1931. MOVEMENT OF WATER IN PLANTS AS AFFECTED BY A MUTUAL RELATION BETWEEN THE PNEUMATIC AND HYDROSTATIC SYSTEMS. Plant Physiol. 6: 495–506, illus.
(8) HAYNES, R.
1930. DETECTING OIL IN TWIGS. Stain Technol. 5: 29.
(9) HERBERT, F. B.
1933. AIRPLANE LIQUID SPRAYING. Jour. Econ. Ent. 26: 1052–1056, illus.
(10) KELLEY, V. W.
1930. EFFECT OF CERTAIN HYDROCARBON OILS ON THE TRANSPIRATION RATE OF SOME DECIDUOUS TREE FRUITS. Ill. Agr. Expt. Sta. Bull. 353, pp. 581–600.
(11) KENDALL, J. C.
1932. OIL SPRAYS. N.H. Agr. Expt. Sta. Bull. 262 (Ann. Rept. 1931): 14.
(12) KNIGHT, H., CHAMBERLIN, J. C., and SAMUELS, C. D.
1929. ON SOME LIMITING FACTORS IN THE USE OF SATURATED PETROLEUM OILS AS INSECTICIDES. Plant Physiol. 4: [299]–321, illus.
(13) PROESCHER, F.
1927. OIL RED O A RAPID FAT STAIN. Stain Technol. 2: 60–61.
(14) WOGLUM, R. S., LAFOLLETTE, J. R., LANDON, W. E., and LEWIS, H. C.
1926–32. HANDBOOK OF CITRUS INSECT CONTROL. Calif. Fruit Growers Exchange Bulls. 3: 6–9, 1926; 4: 27–33, 1927; 7: 35–45, 1930; 9: 14–15, 1932.
(15) YOUNG, P. A.
1930. SPRAY-OIL INJURY IN APPLE LEAVES AND LIMBS. (Abstract) Phytopathology 20: 122.
(16) ———
1931. PENETRATION AND TOXICITIES OF PETROLEUM-OIL SPRAYS. (Abstract) Phytopathology 21: 130.
(17) ———
1934. FUNGI AND BACTERIA AS INDICATORS OF THE EFFECTS OF PETROLEUM OILS ON APPLE LEAVES. Phytopathology 24: 266–275, illus.
(18) ——— and MORRIS, H. E.
1933. INJURY TO APPLE BY PETROLEUM-OIL SPRAYS. Jour. Agr. Research 47: 505–522, illus.

U. S. GOVERNMENT PRINTING OFFICE: 1934