THE EFFECT AND MECHANISM OF ACTION OF OIL AGAINST PLANT DISEASES.

by

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ABSTRACT

A series of narrow distillation range mineral oil fractions and three wide range oils (150 neutral, BP85 and gas oil) were tested against Downy Mildew of Grape Vine, Powdery Mildew of Cucumber and Chocolate Spot of Bean. The relationships between disease control, phytotoxicity and the physical and chemical characteristics of the oils were investigated and the effects of the oils on the pathogens and host plants determined.

In leaf disc experiments the saturated hydrocarbon components of oil fractions gave only slight control of Vine Downy Mildew although in field experiments, BP85 gave between 0 and c. 80% control of artificial infections but was unable to check the progress of heavy natural infection. The unsaturated components gave good disease control but high phytotoxicity, both of which increased with decreasing distillation temperature. Complete control with minimal phytotoxicity was obtained with a deposit of c. 3 mg/100 cm^2 of an oil fraction with a distillation range of 326 to 341°C. Control, which decreased slightly with increasing leaf age and inoculum concentration was also obtained when the oil was applied after infection. It had no direct toxic action on the sporangia or zoospores of Plasmopara viticola and had no effect on the activity of leaf diffusates towards sporangia.

Similar experiments with Cucumber Powdery Mildew showed that both saturated and unsaturated fraction components decreased disease but that the unsaturated component of the most active fraction tested was c. 27 times as effective as the saturated component. Disease control and phytotoxicity increased with decreasing distillation temperature. Increasing conidial inoculum levels decreased disease control which for the unsaturated component was caused by a reduction in hyphal growth, there being no effect on conidial germination at the low oil levels used.

BP85 did not control Chocolate Spot but increased lesion spread by both Botrytis cinerea and B.fabae.

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GENERAL INTRODUCTION

The literature on the effect of mineral oils on plant diseases has been comprehensively reviewed by Calpouzos (1966,1971) and Skellern (1968) and so will not be dealt with in detail. It is worthy of note, however, that although a wide range of diseases has been tested only one, Sigatoka leaf spot of bananas, has been successfully controlled on a commercial scale. The use of oil against this disease was first reported by Guyot and Cuille (1955) and had considerable advantages over conventional fungicides, compared with which it was cheaper, could be applied at very low rates thus reducing labour costs, and was of low toxicity to man and animals. The main disadvantage which emerged later was phytotoxicity and although more highly refined oils and improved spraying techniques helped to reduce this, there are still conflicting reports on the effect of oil on banana yield (Meredith, 1970).

The mechanism of action of oil in controlling plant diseases in general may be either by direct fungitoxic action upon the fungus or indirectly by making the host more resistant. An example of the first case is the control of powdery mildews. Castellani and Matta (1964) showed that oil acted directly by inhibiting germination or growth of quiescent, swelling or germinating conidia and by retarding mycelial growth. Sigatoka leaf spot control provides an outstanding example of the second case. Oil has very little direct effect on the fungus but is able to reduce and delay disease development even after the fungus has established itself inside the leaf (Calpouzos et al., 1959, 1960b). However, the exact mechanism remains to be determined. A third and rather unusual case is the control of wheat stem rust. Rowell et al (1957) showed that oil induced the formation of appressoria by Puccinia graminis var. tritici in non-stomatal areas of wheat

leaves and that these, presumably non-infective appressoria, could similarly be induced when rust spores were germinated on scratched, oil sprayed pieces of plastic. He suggested that on the leaf, indentations such as veins acted in the same way as the scratches, and in the presence of oil induced the fungus to produce non-infective appressoria. Previous work had also suggested that oil may have had an indirect action after penetration (Rowell, 1957).

Apart from papers by Calpouzos et al., (1959,1961 a,b,c), Delfel et al., (1962), Pearson (1958), Price (1960), and Riedhart (1964) all on Sigatoka leaf spot of bananas, there has been very little work published on the relation between type of oil and disease control. The present work is a study of oil types in an attempt to discover other diseases which might be controlled with oil, to correlate the physical and chemical characteristics of oils with fungitoxicity and phytotoxicity, and to investigate the mechanism of action where control seems promising. This has involved the separation of a single crude oil into a series of narrow-cut oil fractions distinguished by distillation range. These fractions have been further separated into saturated and unsaturated hydrocarbon components and these tested for their activity against a number of diseases. Three oils of wide distillation range have also been tested against the same diseases.

The two main diseases investigated have been powdery mildew of cucumber caused by Sphaerotheca fuliginea and downy mildew of vine caused by Plasmopara viticola. Both have been reported to be controlled with oil (Castellani and Matta, 1964,1962), but there has been no further study of the effect of oil type and for the downy mildew, no evidence for a mechanism of action. A third disease, chocolate spot of bean (Vicia faba) has provided a convenient example of disease resistance. Manning (1968)

suggested that the effect of oil in controlling Sigatoka leaf spot might be associated with the induction of a phytoalexin or phytoalexin-like substance. Thus as the phytoalexin involved in chocolate spot has been determined (Letcher et al., 1970), the effect of oil on the disease was studied in the hope that it might provide more evidence for this hypothesis.

MATERIALS & METHODS

A. PLANT MATERIAL

1. Source and cultivar

a) Vine. Vitis vinifera L. "Black Hamburg", a greenhouse cultivar that is susceptible to vine downy mildew was used in early experiments and was originally obtained as propagating material from a vine in the walled garden, Silwood Park, Ascot, Berks., (Skellern, 1968).

"Riesling", a cultivar also susceptible to downy mildew was obtained as seeds from Miss A. Cole, I.C.I. Research Station, Jealotts Hill, Bracknell, Berks. These seeds were originally obtained from grapes in Germany and the seedlings grown from them were used in later experiments when "Black Hamburg" was not available.

b) Cucumber. Cucumis sativus L. "Improved Telegraph", a cultivar that grows well in both cool and warm conditions was used throughout and obtained from Sutton and Sons Ltd., Reading.

c) Bean. Vicia Faba L. "The Sutton", a commercial cultivar, was used throughout and obtained from Sutton and Sons Ltd., Reading.

2. Plant Growth

a) Vine

i) Root-stocks

These were originally propagated from softwood cuttings from Silwood Park (Skellern, 1968). During the winter the root-stocks contained in 10in (25.4cm) clay pots were stored in cold frames. When the sap started to rise in the Spring they were transferred to a cold storage room at c. 5°C while the author was in France conducting field trials. When required they were transferred to a greenhouse to allow bud burst and the resulting shoots then grew rapidly. Fresh material was obtained by

propagating from these plants as described by Skellern (1968). A section of stem about 2in long was taken which included a bud and its subtending leaf. This was pressed into moist peat in a heated propagating case. After roots had been formed the bud burst and a shoot grew out. The cuttings were then potted into 5in (12.7cm) clay pots in "John Innes No.1" potting compost.

ii) Seedlings

As germination was very low, seeds were used in batches of 15 g these being sufficient to give c. 20-30 seedlings. They were treated as follows:-

1) Soaked for 48 h in tapwater.

2) Washed in running tapwater.

3) Sterilized for 5min in sodium hypochlorite (2% available Chlorine).

4) Thoroughly washed again and excess water removed.

5) Vernalized by placing them in sealed Petri-dishes in a refrigerator at c. 3°C for 5 wk. After this time they were sown just below the surface of "John Innes No.1" potting compost in plastic seed trays. Germination occurred in 1-2 wk and seedlings were potted into 3½in (8.9cm) pots after about a month. Plant age varied at the time of use but was usually not less than 2 month from sowing. Those which had grown too large were cut back after which they continued to produce fresh shoots. This technique appeared successful as long as an established root system was present.

One problem encountered was natural infection by powdery mildew. As the vines were required for tests against this disease, it was undesirable to use sprays such as Karathane which might leave a persistent deposit. Initially Deciquam (di-decyl di-methyl-ammonium bromide), a cationic wetting agent produced by the British Hydrological Corporation,

was used, but although it proved effective against mildew, it also caused flecking and shrivelling of leaves, and its use was therefore discontinued. As water sprays were reported by Yarwood (1939) to control powdery mildews, the plants were watered regularly from above so that the leaves were thoroughly washed. Later the plants were removed to the misting section of the college greenhouse at South Kensington. Here they were placed directly under the mist sprayers so that the leaves were kept continually wet. This was discontinued as white deposits, presumably originating from the misting section, built up on the leaves and manual watering was recommenced. Although removed from the direct spray of the misters, the plants were kept in the same section as it was noticed that the vines grew better under these humid conditions.

Although overhead watering kept down the incidence of mildew on the upper leaf surfaces infections continued to appear on the lower surfaces. Those plants which were to be used for downy mildew but not powdery mildew inoculations were therefore treated with Karathane dust using a hand puffer. This treatment effectively controlled the disease.

Finally, infestations of glasshouse red spider mite were kept down by fumigating with azobenzene.

b) Cucumber

Seeds were sown initially in 3½in (8.9cm) pots of "John Innes No.1" potting compost. It was found later, however, that the soil was infested with the larvae of a fungus-gnat which attacked many of the seedlings below soil level, causing them to wilt and die, though older plants seemed less susceptible. Seeds were therefore sown in horticultural vermiculite and watered with Bio liquid plant nutrient (Pan Britannica Industries, Waltham Abbey). Young seedlings were watered with a 1: 600 dilution and older plants with a 1 : 300 dilution. Coarse grade insulating

vermiculite was found unsuitable as it did not provide a sufficiently firm attachment for the roots so the finer grade horticultural vermiculite was used.

c) Bean

Seeds were wrapped in moist tissue paper and germinated in the dark at 20°C. After 4 d healthy seeds with an emergent radicle were sown in "John Innes No.1" potting compost, in 5½in diameter plastic pots, two seeds per pot. The plants were grown in the greenhouse kept at a temperature of 20-25°C with a 16 h photoperiod. Additional lighting was provided by four 80 watt "White" fluorescent tubes suspended about 2 ft (61cm) above soil level. The pots were watered by hand daily until the first leaves emerged from the soil. Thereafter the greenhouse automatic watering system was considered adequate. The plants developed four expanded leaves, 3-4 wk after planting.

3. Experimental Material

a) Vine

i) Whole Plants

Apart from a final test of aromatic fraction phytoxicity, seedlings were only used to maintain the culture of Plasmopara viticola.

ii) Leaf discs

These were cut with a 10 mm cork borer from susceptible leaves of both root-stocks and seedlings. They were floated on distilled water in staining dishes divided by microscope slides into three sections. This arrangement facilitated a direct comparison of treatments on discs of the same leaf whilst making economical use of incubator space. As shown by Skellern (1968) the discs could be maintained on distilled water for up to 4 wk without showing obvious signs of senescence. The use of tapwater or Kinetin caused browing at the edges. In experiments with

Plasmopara viticola the three compartments, each containing five leaf discs, served as three replicates for each treatment. Each replicate was numbered and similarly numbered replicates consisted of discs from the same leaf. Whenever feasible this applied to all replicates but with the small seedling leaves was not usually possible. The size of the discs was restricted to 10 mm as the leaves of seedlings, from which material for the majority of experiments was taken, were much smaller than those from the older root-stocks. This ensured that all treatments were randomized on each leaf, thus compensating for the great variation found by Skellern (1968) in reactions to oil and disease. The lids of the staining dishes could be sealed on with Vaseline petroleum jelly giving *c.* 100% r.h. to ensure maximum sporulation. Unless otherwise stated, experiments were carried out in an illuminated incubator at 20°C.

b) Cucumber

i) Whole Plants

These were used mainly for culturing *Sphaerotheca fuliginea* as they were difficult to spray consistently when testing oils against the disease. They were used however, for testing the effect of oils on plant growth and leaf permeability, and for final tests of fungitoxicity and phytotoxicity of oil fractions. All plants were grown at Chelsea Physic Garden until required, to prevent infection from cultures of *Sphaerotheca fuliginea* maintained on cucumber plants at South Kensington.

ii) Leaf discs

These reduced experimental variation as a large number could be cut from the same leaf, and were very easy to spray and inoculate. They were cut with a 10 mm cork borer from young expanding leaves, and either placed on 0.25% water agar or floated on tap water in plastic Petri-dishes, all treatments in any experiment being randomized on each leaf used.

The number of replicate discs used for each treatment was ten for disease assessment and twenty for chlorophyll measurements. To prevent water condensation on leaf discs, Petri-dishes without lids were placed in germination trays. Up to fifteen dishes in two tiers could be accommodated in each tray. Experiments were carried out in an illuminated cabinet at 20°C.

c) Bean

i) Detached leaves

Detached leaves were used throughout, plants being selected at the four leaf stage. Leaf surfaces were washed thoroughly in a jet of distilled water and allowed to dry. Leaves were detached, their petioles wrapped in moist paper tissue and placed in plastic sandwich boxes (17.5 x 11.5 x 3.0 cm) lined with moist paper tissue. Test tubes were used to support the leaf surfaces in a horizontal position.

B. FUNGAL MATERIAL

1. Origin of Cultures

a) Plasmopara viticola (Bert. and Curt. ex de Bary) de Toni was obtained from Jealotts Hill Research Station.

b) Uncinula necator (Schw.) Burr., was obtained as naturally infected material from the greenhouse at South Kensington.

c) Sphaerotheca fuliginea (Schlecht.) Poll was obtained from Miss A. Cole, I.C.I. Research Station, Jealotts Hill, Bracknell, Berks., identification being based on conidial characteristics as described by Kable ~~et al.~~ and Ballantyne (1963).

d) Botrytis fabae Sard. strain 3 (F3) and Botrytis cinerea Pers. strain 3 (C3) were obtained from J.W. Mansfield, Botany Department, Imperial College.

2. Culture Techniques

a) P.viticola, an obligate parasite was maintained on young vines. Sporangia were collected either by agitating sporulating leaf pieces in distilled water in a test-tube, or by vacuum into a glass side-arm test-tube. In the latter case sporangia were washed down into the tube with distilled water and then shaken to disperse them. Sporangial viability was found to be the same for both methods except that washing often gave better results if the cultures had been kept in dry conditions. Concentrations were obtained by dilution after counts with a haemocytometer. The concentration usually used was 5×10^5 sporangia/ml.

Suspensions were sprayed on to the underside of susceptible leaves using a spray pen (Colour Spray Ltd., North Road, London N.7.) connected to an air cylinder at 5 lb/in^2 (34473.8/Nm^2). The plants were then surrounded by a plastic bag and supported over water to maintain high humidity for 24 h in an illuminated cabinet at 20°C. The bag was removed and the plants left in the incubator for 4 to 5 d when chlorotic areas appeared. The bags were then replaced and left overnight in the dark to induce the formation of sporangia. If sporangia were not required quickly, disease development could be slowed by removing the plants after 2 d to a cold room at 5°C. In this way cultures could be maintained successfully in the leaf for up to 2 wk. Longer periods of storage were not attempted. Sporulating cultures maintained their viability for some days after induction and often two or even three crops of sporangia could be obtained from the same leaf.

b) U.necator, being an obligate parasite was maintained on vine seedlings kept at South Kensington. Fresh plants from Chelsea were infected at 1-2 wk intervals by blowing conidia from infected plants on to young leaves,

infection being reduced on older leaves. Although Delp (1954) found that moisture stress had little effect on mildew development to sporulation, it was found that surrounding infected plants with a plastic bag favoured sporulation.

c) S.fuliginea also being obligate was maintained on cucumber kept at South Kensington. The culture was very easy to maintain and large quantities of conidia could be produced from a few plants since all leaves were equally susceptible. Conditions of high humidity were not necessary for infection or sporulation and fresh plants were inoculated as for U.necator.

d) B.fabae and B.cinerea were both grown on Medium X (Last and Hamley, 1956) in conical Pyrex flasks at room temperature. B.cinerea sporulated well in daylight whereas B.fabae required 'black light' (Philips fluorescent tubes with an emission spectrum of 310 - 410 mu and a maximum emission at 360 mu). Fresh flasks of Medium X were inoculated by adding about 5 ml sterile distilled water to each flask and transfering a small section from a sporulating culture to them. The flasks were then agitated so that the spores were spread evenly over the agar surface.

3. Experimental Techniques

a) Inoculation and incubation

i) P.viticola

Each 10 mm vine leaf disc was inoculated with a single 20 ul drop of a suspension containing 2×10^5 sporangia/ml using an 'Agla' micrometer syringe fitted with a hypodermic needle. Ice cold distilled water was always used to prevent the cytoplasm of sporangia from cleaving

before inoculation. Tubes containing sporangial suspensions were kept in beakers of crushed ice. Inoculations were usually carried out with about 2 to 3 h of the daily light period remaining as inoculations carried out with longer periods of light remaining delayed sporulation. High humidity was maintained for 24 h by placing lids on the staining dishes which were then placed in an illuminated cabinet for 24 h . After this time the lids were removed, the drops taken off with a Pasteur pipette and the discs allowed to dry. The lids were replaced for a further 2 d when they were sealed on the dishes with Vaseline petroleum jelly to ensure c. 100% r.h. for the induction of maximum sporulation.

ii) U.necator and S.fuliginea

Leaf discs were inoculated using settling towers constructed from cardboard and whose diameters varied with the size of the experiment, but were always a minimum of 100 cm high. Petri-dishes containing the discs were arranged in the bottom of each tower so that a maximum gap of 5 cm was left between the dishes and the sides of the towers to allow for edge effects. Before inoculation the discs were always arranged so that each Petri-dish contained discs from each treatment. This ensured that all treatment replicates received the same conidial deposit variation and was necessary since it was found in early experiments that the degree of control obtained with oil sprays depended on inoculum level. Fresh 24 h old conidia were used for each experiment and obtained by blowing off spores from sporulating leaves 24 h before they were required. Plants were then held at the top and to one side of the towers and conidia removed using a hand puffer, and allowed to settle. An attempt was made to ensure that all parts of the tower received similar inoculum levels.

The latter was determined by placing coverslips among the leaf discs and determining the number of conidia/cm^2. Petri-dishes containing leaf discs on agar or floating on water were arranged in germination trays as described before and incubated in an illuminated cabinet at 20°C.

iii) B.fabae and B.cinerea

Suspensions of spores of both fungi were prepared by adding about 50 ml of sterile distilled water to 10 - 15 d cultures of both fungi. The sporulating mat of each culture was gently scraped with a sterile inoculating loop and the resulting suspension filtered through four layers of muslin to remove hyphal fragments. The spore suspension was washed three times with sterile distilled water by centrifuging at 850 g for 1-2 min. Unless otherwise stated spore concentrations of both fungi were made up to 5×10^5 spores/ml after haemocytometer counts.

The inoculation technique used was essentially similar to that used by Deverall (1960). Ten drops (10 µl/drop) of spore suspension were placed on the upper surface of each leaflet by means of an 'Agla' micrometer syringe fitted with a hypodermic needle As the large spores of B.fabae tended to settle quickly the suspension was stirred by introducing an air bubble into the barrel of the syringe which was rotated after every five drops. After inoculation, the boxes were closed using tightly fitting lids with pieces of moist tissue adhering to their lower surfaces which, as shown by Skipp (1970) prevented the formation of large water drops which might fall onto the leaves. The boxes were incubated in an illuminated cabinet at 17°C.

b) Release of powdery mildew conidia from leaf discs

A standard technique was designed to measure the effect of oil

sprays on the release of conidia from sporulating leaf discs. The development of the technique is described in Experimental Section III, only the final arrangement being described here.

Discs (10 mm diameter) were carefully cut from sporulating leaves with a cork borer and floated on tap water. When required they were placed on paper tissue to dry the underside of each disc A pin was then gently inserted through the centre of the disc and used to carry and attach the disc to a piece of plasticine attached to a wooden block (2.5 x 7.5 x 6 cm) as shown in Figs. 1a and 1b. The block was held at an angle of c.20° to the vertical with a Pasteur pipette held firmly at a distance of 1.5 cm vertically above the disc. The pipette and block were then moved into a position adjacent to a cut-away section at the top of a settling tower (8 x 75 cm) as shown in Figs. 1c and 1d. Conidia were then released by two sharp puffs from the Pasteur pipette. They were retained in the tower by the opposite wall and settled on to a coverslip at the bottom.

Four replicates of eight discs were used for each treatment and 100 conidia were counted on each coverslip. Results were expressed as conidia/cm^2.

c) Spore germination tests

i) P.viticola and Botrytis sp.

Spore germination tests with these fungi were carried out on new, glass slides cleaned as follows:-

1) Slides thoroughly washed in hot water and Stergene, a nonionic detergent.

2) Rinsed in hot tap water followed by distilled water to remove detergent.

3) Allowed to stand in glass distilled water for 48 h.

4) Drained, soaked in Analar acetone for a few hours and finally drained and dried in a hot oven.

Fig. 1a. Wooden block with leaf disc attached

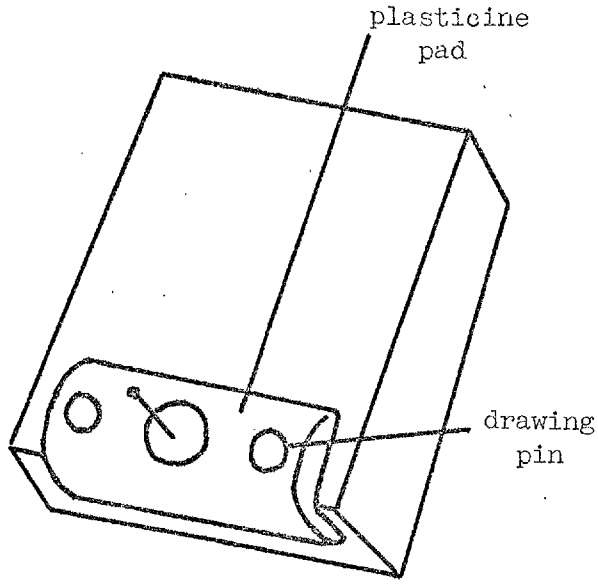


Fig. 1b. Side view of block

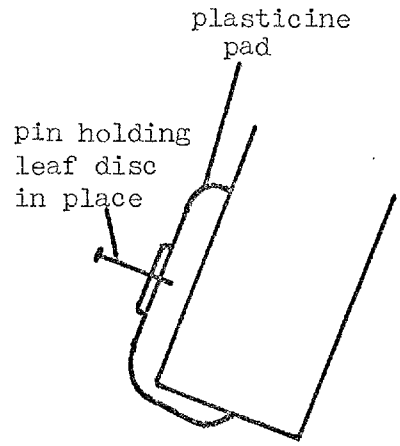


Fig. 1c. Settling tower showing cut away section

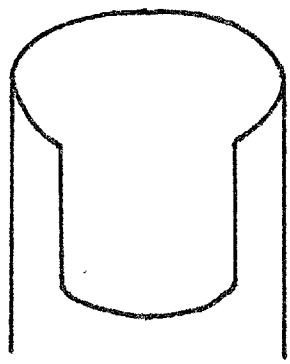
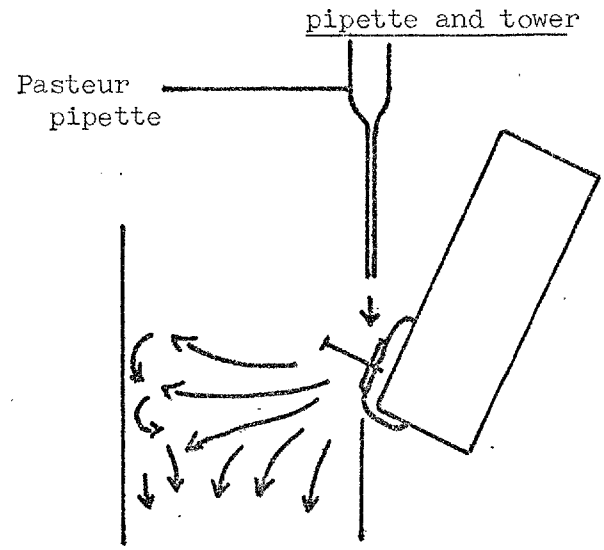


Fig. 1d. Sectional view of block pipette and tower



The exclusion of the conventional laboratory glassware cleaning chemical, chromic acid, was recommended by Deverall (1965) who found its use undesirable in experiments on the stability of IAA, which still contaminated glass slides after copious washings in hot tap water and glass distilled water. Smith (1967) found both Decon 25 and Chemico unsatisfactory as cleaning agents, the former being ineffective in removing material adhering to slides and causing drops to spread excessively, the latter being difficult to remove from slides. The procedure described above has been found by other workers in the Botany Department, Imperial College, to give satisfactory results in germination tests with a number of different fungi.

Three 20 µl drops of sterile distilled water (ice cold for P.viticola) were placed on the slides and to each was added a 2 µl drop of a suspension of 5×10^5 spores of Botrytis sp/ml or 4×10^5 sporangia of P.viticola per ml of ice cold water (to prevent cytoplasmic cleavage). The slides were placed in sandwich boxes (17.5 x 11 x 3.0 cm) lined with moist paper tissue and supported in rows of four to six by two test tubes. As described for the incubation of Botrytis sp., care was taken that the lid of each box fitted tightly and was also lined with moist tissue. Both fungi were kept in the dark at 17°C for 24 h. For each of six drops 200 spores or sporangia were counted. Germination for P.viticola was given by the number of empty sporangia and for Botrytis spp, the presence of a recognisable germ-tube of any length.

ii) Powdery mildews

New coverslips (18 mm x 18 mm) were hand cleaned by rubbing them without any cleaning agent between first finger and thumb under running tap water. They were then rubbed between two layers of paper tissue until they felt completely smooth. This method of cleaning was found

by R.G. Perera (personal communication, Imperial College, 1970) to give good results in germination tests with a wide range of powdery mildew species. Coverslips were dusted with 24 h old conidia produced as for leaf disc inoculations using an inoculation tower. With S.fuliginea greater germination was obtained by using spores from lightly infected leaves on which colonies were scattered and from which old conidia could be removed. With more heavily infected leaves the hairs made complete removal of old conidia difficult, even when the leaves were blown violently.

Coverslips bearing conidia were quickly removed from the tower and placed in humidity chambers as described by Manners and Hosain (1963). These consisted of staining blocks containing distilled water and transparent glass lids with two small fragments of glass stuck on to the under surface of each lid with Araldite or parraffin wax. The coverslips with conidia were stuck on to the fragments with Vaseline petroleum jelly so that on assembly of the chambers, the coverslips were suspended within a few mm of the surface of the water with the conidia in the narrow space between the lid and coverslip. This prevented water condensation round conidia which inhibited germination.

Germination was determined after 24 h when the lids of the humidity chambers were removed and the coverslips placed on microscope slides. For each of four replicate coverslips a total of 100 non-contacting conidia were counted, the criterion for germination being taken as the presence of a recognisable germ tube of any length. Conidia in groups were not counted as these were found by R.G. Perera (personal communication, Imperial College, 1970) to give poor germination.

d) Examination of fungi in vivo

i) Chlorine bleach

The technique of Janes (1962) was used in an attempt to study the behaviour of zoospores of P.viticola on vine leaf discs. Chlorine was

prepared from a mixture of potassium permanganate and concentrated hydrochloric acid. The gas was passed through water to remove acid and directed on to 5mm discs cut from the inoculated area of 10 mm leaf discs and resting in a Petri-dish. The discs bleached most rapidly when moist on their undersurface but not floating on water or resting on moist filter paper. After bleaching, the discs were treated with ammonia vapour to remove excess chlorine and stained and mounted in a phenolic solution of acetic acid-aniline blue (Jones and Mollison, 1948) and examined under high power and oil immersion objectives.

ii) Preece technique

This technique of Preece (1965) as used for vine by Royle (1971) was also used to study the behaviour of zoospores of P.viticola on leaf discs. A 5 mm disc was cut from the inoculated area of a 10 mm leaf disc and treated as follows:-

1) Fixed and decolourized in ethanol.
2) Warmed in a mixture of ethanol and acetic acid (1:1) for a few minutes.
3) Stained for 2-3 min in phenol-acetic-aniline blue (Jones and Mollison, 1948).
4) Rinsed in 80% ethanol to differentiate fungal material.
5) Transferred to ethanol.
6) Mounted in Euparel Essence.

Mounts were examined under high power and oil objectives.

iii) Celloidin peel

This method was essentially the same as that of Wenzl (1939) and was used for studying germination and growth of germ tubes of S.fuliginea on cucumber leaf discs and the germination of sporangia of P.viticola on vine leaf discs. Celloidin wool (Griffin and George Ltd) was made up

to 5% in a mixture of absolute alcohol and ether (3:1). This was smeared on the infected surface with a thin glass rod, allowed to dry for 30 to 90 min, depending on the thickness of the film, peeled off and stained in 5% aniline blue in lactophenol and mounted in lactophenol. All fungal structures outside the cuticle were removed by the peel which also contained impressions of plant hairs and epidermal cells. The preparations could be kept for many months by occasionally replenishing the lactophenol. A second peel was always taken from each leaf disc to ensure that all conidia had been removed.

e) In-vitro bioassay of oils

i) Agar diffusion

The technique was essentially that of the filter-paper disc method described by Vincent and Vincent (1944) and modified by Maruzzella and Liguori (1958) for testing the anti-fungal activity of essential oils. Further modifications included the low temperature pre-treatment of seeded plates and the use of diluted agar medium.

Plates of Botrytis cinerea spores seeded in 50% Czapek-Dox nutrients in 1.2% agar were prepared as follows. A dense suspension of spores was prepared by adding 10 ml aliquots of sterile distilled water to sporulating cultures of B.cinerea on Medium X (Last and Hamley, 1956). The surface of each culture was gently rubbed with a sterile inoculating loop, the flask shaken and the resulting suspension poured into a sterile bottle. Using a sterile pipette, 0.5 ml aliquots of suspensions were added to tubes of sterilized and melted agar maintained at c.45°C in a water bath. After mixing rapidly the agar in each tube was poured over a basal layer of 7.5 ml solidified agar in a 9 cm Petri-dish. The seeded plates were then kept c. 3°C until required.

The oil samples were tested as follows. Filter paper discs (10 mm, Whatman No.1), sterilized by autoclaving at 15 lb/in^2 (103421.4 Nm^2) for 15 min, were soaked in the oils under test, excess oil removed by

draining for a few seconds, and placed in the centres of seeded plates. These were then kept at c. 3°C for a further 4 h and then removed to an incubator at 25°C. Fungitoxicity of the oils was measured at 24 and 48 h as the width (mm) of the clear zone of growth inhibition around the paper discs. Four replicate plates were used for each treatment.

ii) End-point technique

This was essentially the method described by Barnes (1963) for testing the toxicity of vegetable oils to Fusicladium effusum. Discs (10 mm) were cut from both mycelial (4 d on Medium X) and sporulating (10-15 d on Medium X) cultures of B.cinerea and treated with oil as follows. Three discs were added to about 5 ml of test oil (sterilized by millipore filtration) in sterile vials covered and shaken vigorously to dislodge air bubbles. After varying periods of immersion the discs were removed, drained of excess oil, and placed equidistantly on an agar plate. Growth on and out of the discs was recorded as + or - or + (sli) (=slight).

C. DISEASE ASSESSMENT

1. P. viticola

Sporulation on leaf discs was estimated by both visually grading sporulating lesions and by washing off and counting the number of sporangia produced.

Grades were on an arbitrary scale from 0 to 5 as shown in Table 1.

Table 1 P. viticola sporulation-visual grades

0	nil
0.5	trace
1.0	sparse
2.0	sparse-light
3.0	light
4.0	moderate
5.0	heavy

By continually comparing replicates fairly reliable grade estimates could be obtained though grade values were not necessarily comparable in different experiments.

Sporulation counts were made by agitating the five replicate discs in 0.5 ml 20% (v/v) ethanol in a small glass tube (1 x 5 cm) using a "Whirlymixer". Agitation was continued until all sporangia were removed and the resulting suspensions counted with a haemocytometer; the results are expressed as the number sporangia/disc.

As shown by Malcolmson (1969) for Phytophthora infestans the ethanol preserved the sporangia and their contents for many weeks and even after 4 months preservation was still good.

2. Powdery mildews

a) Leaf area cover

Disease incidence was estimated in terms of the percentage leaf area covered with mycelium. As it was often difficult to identify infected areas clearly because of leaf senescence, discs were first decolourized in ethanol, stained in phenol-acetic-aniline blue (Jones and Mollison, 1948) and rinsed and stored in distilled water. The discs were then scored for mycelial coverage using standard area diagrams based on the Horsfall-Barratt Grading System (Horsfall and Barratt, 1945) and shown in Fig. 2. This system is based on the Weber-Fechner Law which states that the human eye distinguishes according to the logarithm of the light intensity. Thus grades are based on equal ability to distinguish, not on equal disease. Below 50% the eye sees the amount of diseased tissue and above it the amount of disease free tissue. Taking 50% as mid-point, grades differ by a factor of two in each direction as shown in Table 2.

Fig. 2. Standard area diagrams based on the Horsfall-Barratt (1945) grading system

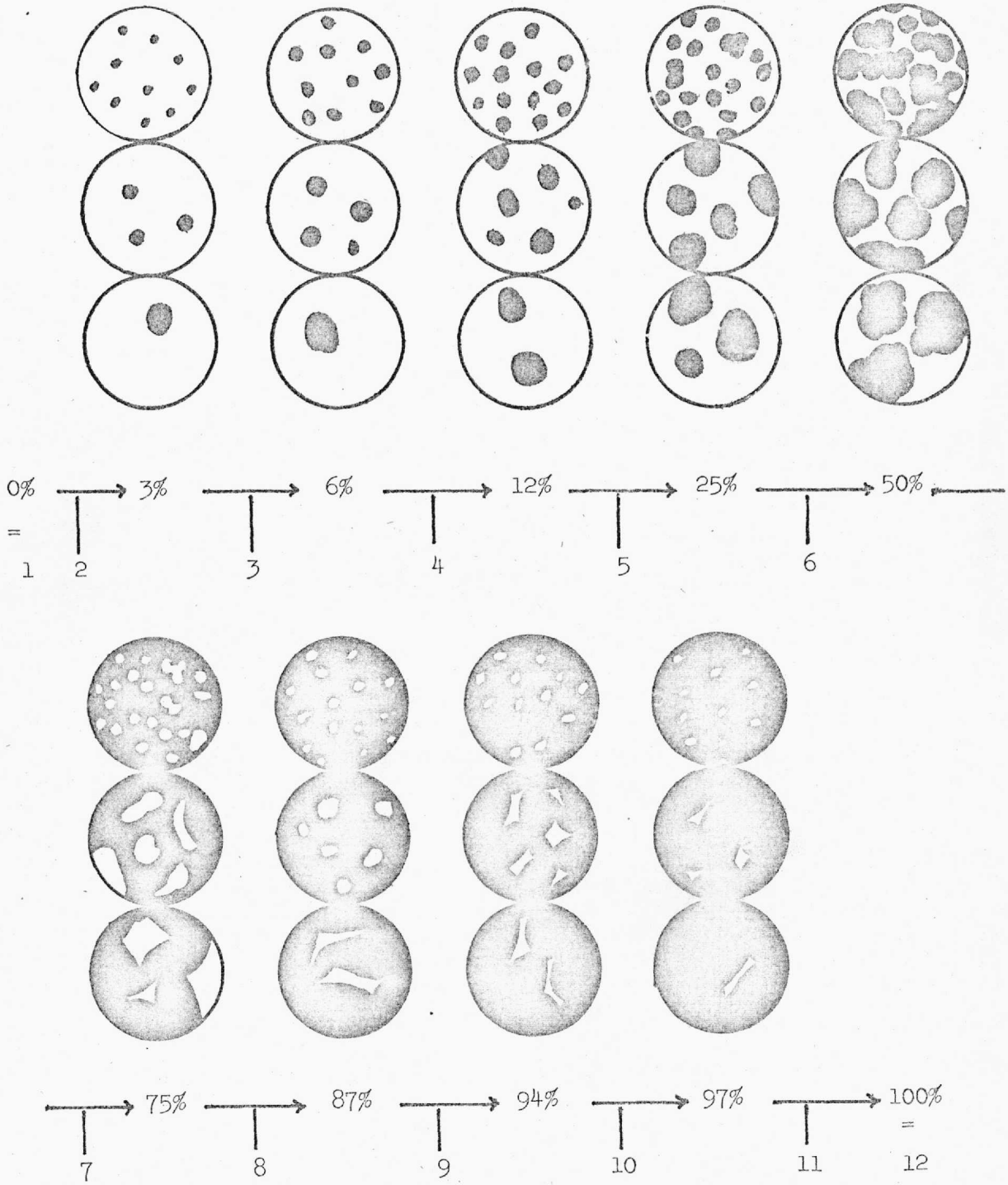


Table 2. The Horsfall-Barratt Grading System

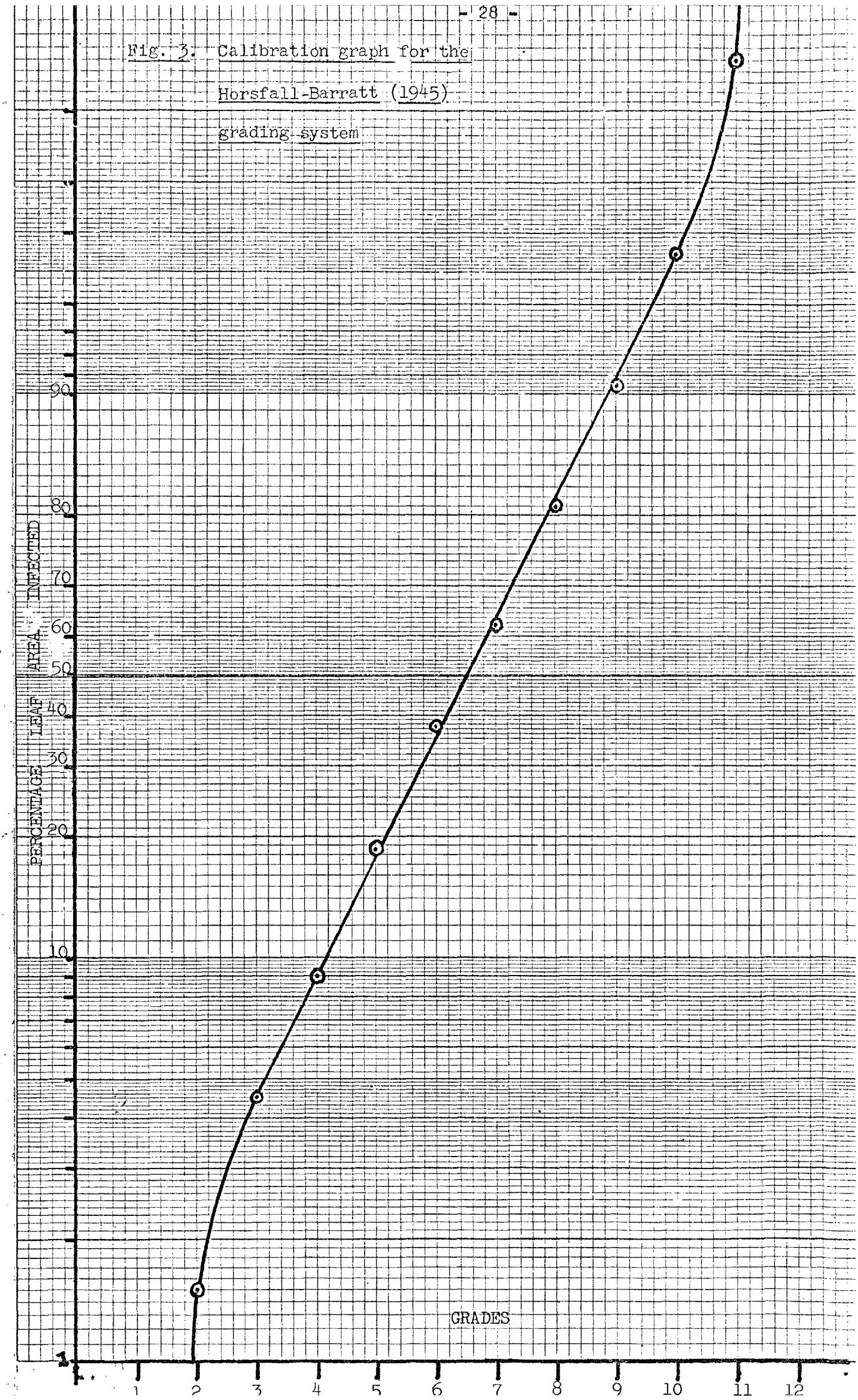
Disease Grade	Percentage Disease Cover
1	0
2	0-3
3	3-6
4	6-12
5	12-25
6	25-50
7	50-75
8	75-87
9	87-94
10	94-97
11	97-100
12	100

A calibration curve is set up (Fig. 3) with grade numbers on the X-axis and percentage disease cover on a special semi-legarithmic Y-axis with one and one half phases from either end up to 50%. This was constructed from two pieces of two phase logarithm paper by cutting both at the one and one half phase point and joining them together. The Y-axis consists of the mid-points of the percentage disease cover range for each grade. This produces a straight line between grades 3 and 10 i.e. mid-points of 4.5% and 95.5%. At either end the line curves away to 1.0 and 99.0%. The mean grade for each treatment is given by the total grade readings divided by the number of readings. Statistical analysis is restricted to grades as these are theoretically of the same accuracy as opposed to percentages which are not.

b) Sporulation

This was measured in terms of the number of conidiophores/cm^2 using a binocular stereo-zoom dissecting microscope with an eye-piece graticule divided into 100 x 1 mm squares.

Fig. 3. Calibration graph for the Horsfall-Barratt (1945) grading system



Sporulating leaf discs were fixed and stained as for leaf area cover estimations after having removed clumped conidia. The latter if left on the discs made counting difficult. Each disc was traversed along two diameters at 90° to each other counting in consecutive fields.

In some cases sporulation was measured as the number of conidia released by the technique used for the release of conidia from sporulating leaf discs.

3. Botrytis sp.

Lesions (= chocolate spots) were counted and expressed as a percentage of total inoculations.

Lesion spread was roughly graded in the first experiment as follows:-

a = slight spread
b = moderate spread
c = coalescence of lesions

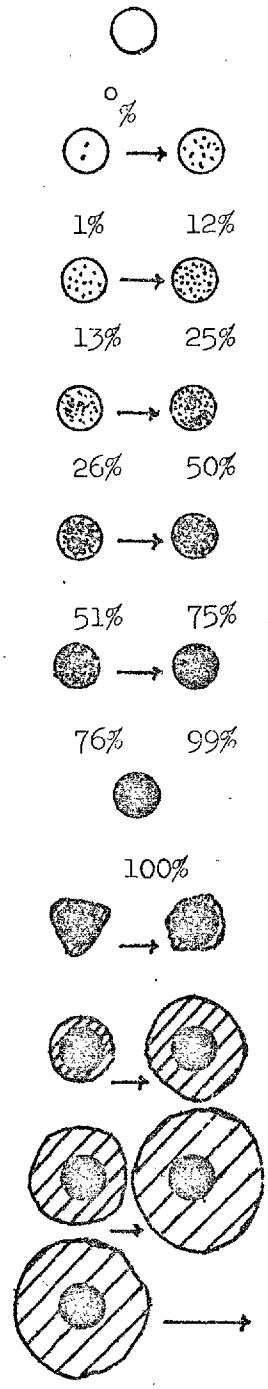
In all other experiments lesions were graded on a scale devised by J.W. Mansfield (Botany Department, Imperial College) and illustrated in Fig. 4. Lesion grades up to F+++ were roughly based on the Weber-Fechner law, as in the Horsfall-Barratt grading system.

D. OILS

1. Wide Distillation Range Oils

These were typical of those used commercially as pesticide oils and are highly refined, complex mixtures of hydrocarbons. These are almost entirely the fully saturated paraffins (straight chains) or naphthenes (cyclic) though small amounts of unsaturates (aromatic and heterocyclic hydrocarbons) are usually present.

Fig. 4. "Chocolate Spot" grading system



Spray oils are selected on the basis of certain chemical and physical properties, the more important ones being as follows:-

1) Distillation temperature at the 50% point and temperature range between 10 and 90% of the distillation. These give an indication of molecular size, the lower the temperature, the smaller the molecules.

2) Unsulphonated residue index (U.R.), high values indicating a high degree of refinement or low unsaturate content.

3) Gravity or density.

4) Viscosity.

The latter is related to distillation temperature, low values indicating low temperature ranges.

For comparative purposes oils must be taken from the same crude oil source for it is quite common for oils from different sources to have similar viscosities but different distillation temperatures. Thus Calpouzos et al. (1961) reported that a naphthenic oil caused less phytotoxic flecking on banana leaves than paraffinic oil of the same viscosity. As pointed out by Chapman et al. (1962), however, the two oils were from different sources and had different molecular structures with the paraffinic oil having a higher distillation temperature than the naphthenic oil, and thus being a heavier and more persistent oil. On the basis of this, one would naturally expect the paraffinic oil to be more phytotoxic. The importance of spray oil source was confirmed by Calpouzos (1964) when comparing naphthenic and paraffinic oils from different sources on the basis of the same distillation temperatures. No significant differences were found for disease control or phytotoxicity between the two types.

Specifications of the oils used are given in Table 3.

Table 3 Specifications of wide distillation range oils.

Oil	Gas oil	BP85	150 Neutral
Distillation at atmospheric pressure (°C)			
50% point	265	392	428
10-90% range	107 (216-323)	29(381-410)	42(409-451)
0-100% range	167 (185-352)	79(360-439)	116(372-488)
Unsulphonated residue (U.R.)	79%	94-96%	c. 93%
Viscosity at			
21.1°C	4.14 cSt	32.19 cSt	73.28 cSt
37.8°C	2.86 cSt	16.13 cSt	32.22 cSt
100°C	1.14 cSt	3.406 cSt	5.22 cSt
Specific gravity 15.5°C/15.5°C	0.8350	0.8480	0.8770

Gas Oil was used as a highly volatile, low boiling point oil to compare with BP 85 a low volatile, high boiling point oil. The oil, 150 Neutral had a similar (though slightly higher) distillation range to BP 85, but a much higher viscosity. This is due to the rapid increase in viscosity with increased distillation temperature in this range (Appendix 1b).

2. Experimental Oil Fractions

a) Distillation procedure

The fractions were obtained from a Kuwait crude oil using a packed distillation column incorporating raschig rings equivalent to a theoretical 14 plate column (Sunbury Report No. 10053). The distillation was carried out by the Crude Oil Assay Group, Analytical Section, British Petroleum Research Centre, Sunbury-on-Thames, Middlesex, a general description of the stages being given below.

The distillation was started at atmospheric pressure and continued up to an overhead temperature (O.H.T.) of 178.5°C. The pressure was then reduced to 100 mm mercury and distillation continued to an O.H.T. of 266°C followed by 2 mm mercury up to an O.H.T. of 407°C. The base temperature range during the distillation was 275°C to 320°C which for a 14 plate still, was low enough to prevent oil cracking. At a pot temperature of 340°C the oil was just beginning to crack and the distillation was stopped. The distillation data are given in Table 4.

Table 4 Experimental oil fraction distillation data

Reference No.	Fraction No.	Boiling Range in °C (Atmospheric pressure)	% Weight	Volume (ml)
	Gas	15	2.00	200
	Gasoline	15-150	14.10	"
14A/740	9	150-167	2.10	"
	10	167-181	2.15	"
	11	181-193	2.15	"
	12	193-210	2.20	"
	13	210-224	2.20	"
	14	224-238	2.20	"
	15	238-252	2.25	"
	16	252-264	2.30	"
	17	264-282	2.30	"
	18	282-297	2.35	"
	19	297-312	2.35	"
	20	312-326	2.35	"
	21	326-341	2.35	"
	22	341-357	2.40	"
	23	357-374	2.40	"
	24	374-392	2.45	"
	25	392-413	2.45	"
HC3/811	1	413-421	1.00	20
	2	421-433	1.00	"
	3	433-443	1.05	"
	4	443-453	1.00	"
	5	453-462	1.05	"
	6	462-469	1.05	"

cont./

Table 4 Experimental oil fraction distillation data cont.

Reference No.	Fraction No.	Boiling Range in °C (Atmospheric pressure)	% Weight	Volume (ml)
	7	469-474	1.00	20
	8	474-480	1.05	"
	9	480-488	1.05	"
	10	488-495	1.00	"
	11	495-498	0.45	"

b) Extraction procedures

The fractions were separated initially into two hydrocarbon components:-

1) A saturated hydrocarbon component (N+P) consisting of straight chain paraffins and alicyclic naphthenes.

2) An unsaturated hydrocarbon component (A+H) consisting of aromatic (homocyclic) and heterocyclic compounds.

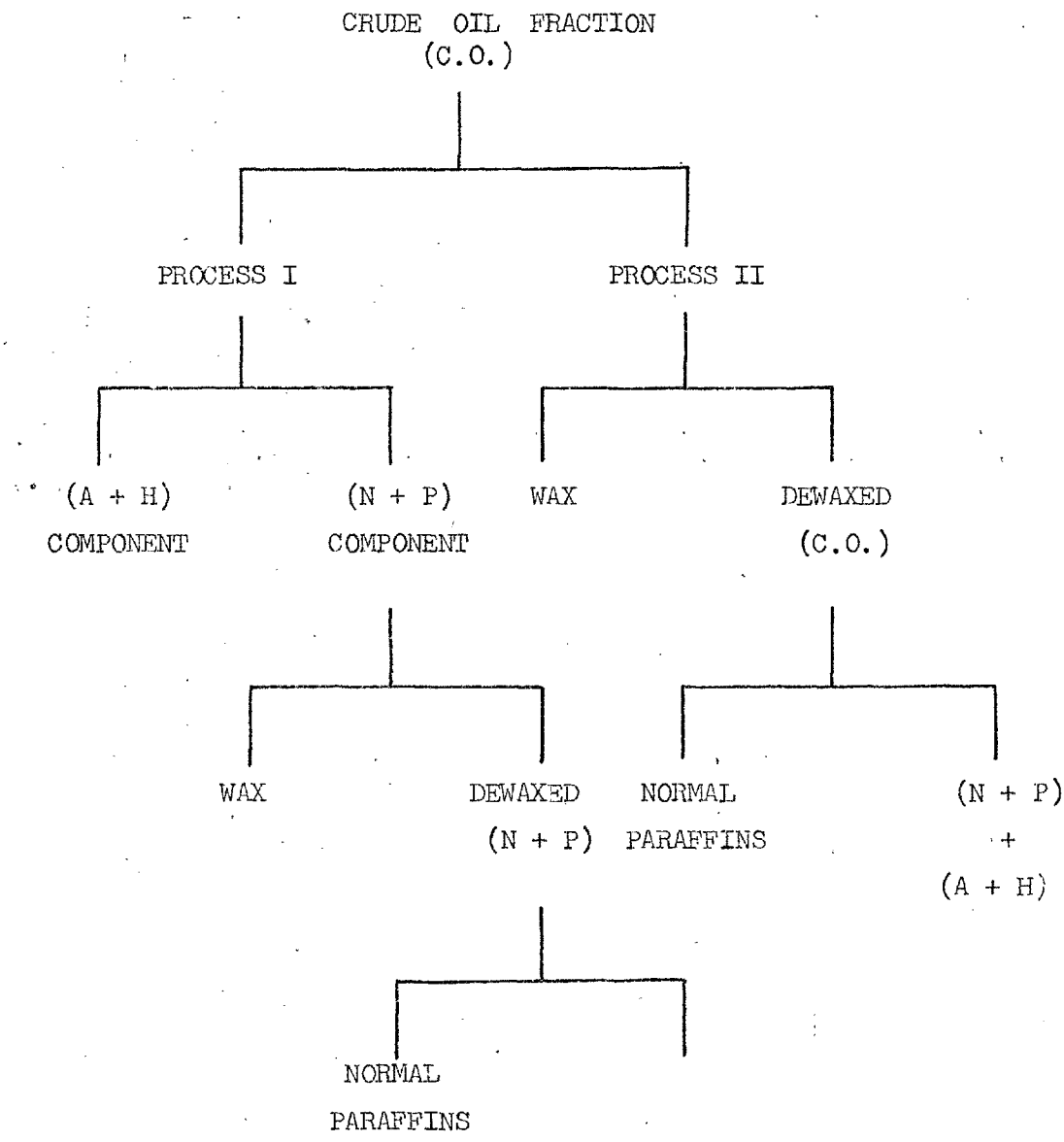
The saturated fraction was then extracted for normal paraffins and waxes which in the case of the higher boiling point fractions were solid at normal room temperature. In a second series of extractions normal paraffins and waxes were extracted directly from crude oil fraction samples. The complete extraction scheme is shown in Fig. 5.

i) Separation of aromatics

Aromatic and heterocyclic compounds were removed from the oil fractions by adsorption onto silica-gel using pentane as the elutant for (N+P) components and absolute alcohol for (A+H) components. This process differs from that used for commerical spray oils used to control banana leaf spot. The latter are refined by solvent extraction which, although relatively cheap, does not completely remove unsaturates.

The quantity of silica-gel required for each extraction was calculated on the basis of twenty times the expected weight of unsaturates

Fig. 5. The complete crude oil fraction extraction scheme



in the sample. In early extractions this was assumed to be 20% of the crude oil fraction sample. Further work showed that this was too low, some of the higher boiling point fractions containing over 40%. Thus, in early extractions, the silica-gel was overloaded and complete separation was not attained. The success of the extraction could, however, be checked by mass spectroscopy of the resulting (N+P) fractions from which the presence of unsaturates could be detected. The volume of pentane was about twenty times the weight of the (N+P) component and the volume of ethanol about 100 times the weight of the (A+H) component.

The silica-gel was activated in an electric oven at 250°C for 3 to 5 h and left overnight to cool in an airtight container. The activated gel was then poured into a glass extraction column with a sintered glass plate and valve at the bottom and a litre reservoir at the top. As the gel was poured in, a Pifco vibrator was used to ensure that the gel was packed evenly. The size of the column used varied but usually held between 500 and 700 g gel.

The oil was carefully poured into the columns and allowed to move down until the level was just above the gel. Redistilled pentane was then used in small volumes (about two volumes of 25 ml/90 g crude oil) to wash out the flask used to weigh the oil, and added to the column to dilute the oil. The oil was again allowed to move down the column until just above the gel, when the rest of the pentane was added. The eluted (N+P) component in pentane was collected in a flask at the bottom. The column was left until the level of pentane was just above the gel at which time the ethanol was added to the top of the column to elute the (A+H) component. The solvent front whose position could be checked

by detecting by hand the heat produced at the front, was allowed to pass down the column until about one half to two thirds of the way down. At this time the collecting flask was changed, the (N+P) component having by this time passed off the column.

The pentane and ethanol were removed from the (N+P) and (A+H) components respectively under reduced pressure in a rotary evaporator heated in a water bath at c. 70°C. The final weights of components were determined and thus the percentage (N+P) and (A+H) in the original crude oil fractions calculated.

ii) Separation of wax and determination of 'wax content'

Wax is defined with reference to the conditions under which it is separated from its parent material. 'Wax content' is an empirical value and in the method described below is given by the percentage by weight of the material precipitated when a solution of the asphalt free product in methylene chloride is cooled to -25°F (-31.7°C). The extraction and determination of 'wax content' were as laid down by the British Petroleum Research Centre Methods of Testing Vol.I 'Wax content of Petroleum Products'.

The sample of oil was dissolved in methylene chloride (10 ml/g sample) warmed over a bunsen burner until just boiling. The flask containing the mixture was then immersed in a cooling bath maintained at -30°F (-34.5°C). The mixture was allowed to cool to -31.7°C and maintained at this temperature for 30 min with frequent shaking. The precipitated wax was separated off by suction through a cold sintered glass filter funnel taking care that the filter did not run dry with the consequent caking of wax. Moisture condensation inside the funnel during cooling was prevented by suitably covering the top and care was

taken that the level of mixture in the funnel was at no time higher or as high as the level of the cooling medium surrounding the funnel (Solid CO_2). The whole contents of the flask were transferred to the filter and the flask and thermometer washed thoroughly with a little methylene chloride chilled to -31.7°C . The washings were passed through the filter and the washing repeated until the flask and small quantity of wax adhering to it were free from oil.

The wax on the filter was washed with methylene chloride at -31.7°C until the wax was white or not more than slightly yellow in case of residual material. During washing the wax was covered with liquid until free from oil to prevent compacting and the formation of cracks in which case it would not be possible to wash out the oil. The wax was then allowed to warm to room temperature and then washed off with carbon tetrachloride. The latter was distilled off and the wax dried at 105°C and cooled for 1 h in a desiccator (> 15 min and < 1 h).

iii) Separation and determination of normal paraffins

These were extracted using a 5 A° molecular sieve, the weight of sieve used being ten times the percentage estimated normal paraffins. To ensure complete separation the estimate was taken as 10%.

The oil was dissolved in benzene and refluxed with the sieve for about 8 h. The normal paraffins were thus adsorbed by the sieve and were desorbed by washing with n-heptane in a Soxhlet apparatus. The n-heptane displaced the normal paraffins which were regained after the excess n-heptane was distilled off. Oil minus normal paraffins was obtained by distilling off the benzene.

3. Determination of Physical Constants of Oils

a) Viscosity

Oil viscosity was measured in Cannon-Fenske size 200 viscometers suspended in a water bath at 100°F (37.8°C), this being a standard temperature for estimating the viscosity of petroleum products. The test oils were expected to show a wide range of viscosities and therefore standard flow times for each viscometer were measured using 10 ml of three standard oils (gas oil, BP 85 and 150 neutral). These covered a wide viscosity range, the standard used when calculating test oil viscosity being that which had the nearest flow time to the test oils.

When the volume of oil sample was insufficient to use with a viscometer, rough estimates were made by timing the flow of 0.05 ml of oil down a 0.1 ml pipette (Blanbrand Colodur W.G.) at room temperature, c. 20°C . The pipette was held in a clamp together with a 100°C thermometer to measure room temperature. To ensure standardization, the pipette was washed before use and after every oil in Analar petroleum ether, the same treatment being given to a second thermometer. Both were then placed in a hot oven until the thermometer temperature reached 19°C when they were removed and left in a clamp for 5 min to equilibrate with room temperature. About 0.15 ml of oil was then sucked up to about 0.05 ml above the beginning of the pipettes calibration markings. Holding the oil at this level, the pipette was secured in a clamp above a collecting tube. The oil was then allowed to fall and the time taken for the level to reach the 0.05 ml mark. Readings were repeated until consistent values were obtained.

b) Volatility

The method used for measuring oil volatility was similar to that used by Delfel et al. (1962) when studying the relationship between

banana leaf spot control and spray oil volatility. Oil volatility at 25°C was determined by measuring its evaporation from filter-paper over extended periods of time.

Oil was sprayed onto the surface of a Whatman No.1 filter-paper disc (11.0 cm) using the Potter spray tower. The filter-paper was weighed immediately before and after applying the oil and at various times up to 30 d. Blank discs were included to correct for moisture gained or lost by the filter-paper with changes in humidity. Each disc was suspended by a metal hook inserted through a small hole punched in each disc and hung from a rod in a draught free area of a constant temperature room. Each determination was run in triplicate and the volatility of the oils calculated as the percentage weight loss.

4. Application of Oils

In most laboratory experiments oils were applied using a Potter spray tower as described below. During field work in France ultra-low-volume atomizers were used. These were designed to operate at rates of 10 l/ha. and below.

a) Potter spray tower

This was originally designed by Potter (1941,1952) for testing insecticides and is capable of giving deposits down to 1 mg/100 cm^2, which although varying with different crops is equivalent to about 11 l/ha (c. 1 gal/ac) or below. The tower used in the present work was manufactured by the Burkard Manufacturing Co. Ltd., Rickmansworth, Herts., as the ST-4 Laboratory Spray Tower and is the same as the one used by Skellern (1968).

It is essentially an atomizing nozzle centred over a tube with a cone shaped upper portion (34 x 16 - 12 cm) and a parallel sided lower

portion. The oil to be sprayed is introduced into a reservoir close to the nozzle. Air from a compressed air cylinder is allowed into a pressure chamber at the base of the tower by an on/off switch incorporated into the tower base and between the pressure chamber and air cylinder. The build up of pressure in this chamber then lifts a spray table to a position 2.5 cm from the base of the tube. The pressure in this pipe can be adjusted by a valve and is registered on a dial on the base of the tower. The atomizing nozzle is in the form of a tapering double pipe, the air passing out of the outer pipe thus creating a suction in the inner pipe and sucking the oil out of the reservoir to which the inner pipe is connected by a short tube. A more detailed description of the development and design of the tower is given by Potter (1941, 1952). Each spray was performed separately, as Skellern (1968) found that even if the tap was closed before all the spray was delivered, the spray did not stop because a siphon was created which pulled the oil into the nozzle. The whole tower was enclosed within a polythene tent supported by a rectangular wooden frame.

For small volumes it was found more convenient to modify the spraying procedure slightly. Instead of a reservoir, a one ml blow-out pipette containing the required amount of oil and with a suitably fine tip was introduced into the top of the inner pipe of the spray nozzle. This technique was particularly convenient for oils of high viscosity and also for spray volumes below 0.1 ml.

The tower was calibrated by spraying known volumes of oil on to previously weighed circles of aluminium foil and then reweighing. The results for different oils are given in Appendix Id. The deposit rate

in mg/100 cm^2 could then be transformed for vine into spray rates of litres per hectare using results obtained during the field trials in France 1969, with the minimicron. No equivalent results were obtained for cucumber under commercial spraying conditions.

Oil deposits on leaves and cotyledons were determined by carefully weighing them before and after spraying as follows. The leaf or cotyledon was cut and the petiole sealed with vaseline and transferred to an accurate single pan balance. One minute after cutting the weight was taken. The leaf or cotyledon was then carried on a plastic spray platform and placed in the bottom of the Potter tower and sprayed. They were then returned to the balance and after waiting 90 s from the first weighing, a second reading was taken, followed by another at 2 min and thereafter at 1 min intervals up to 20 min . This standardization of weighing times helped to allow for weight losses due to water loss. Unsprayed leaves and cotyledons were used to correct for this weight loss. Results were expressed in terms of mg/100 cm^2.

b) Spraying

i) Vine

All experiments involving the spray tower were carried out on leaf discs. These were arranged on moist paper tissue in a 13 cm Petri-dish so that the pressure of the spray did not dislodge them. After spraying they were replaced on water or agar in Petri-dishes.

ii) Cucumber

Apart from their use in calibrating the Potter spray tower as described above, detached leaves were not used. All experiments involving the spray

tower were carried out on leaf discs which were handled in the same way as for vine.

iii) Broad Bean

Detached leaves were used in all experiments, the right hand leaflet being sprayed with the other as control. The left hand leaflet was covered by enclosing it in a plastic Petri-dish as shown in Fig. 6. The dish had a gap cut in the side of the lower dish, the upper dish enclosing the petiole and left hand leaflet. To prevent oil entering through the gap, a cotton wool plug was used. The dish containing the leaf was carried and placed at the bottom of the spray tower on a transparent plastic base.

iv) Spore germination test slides

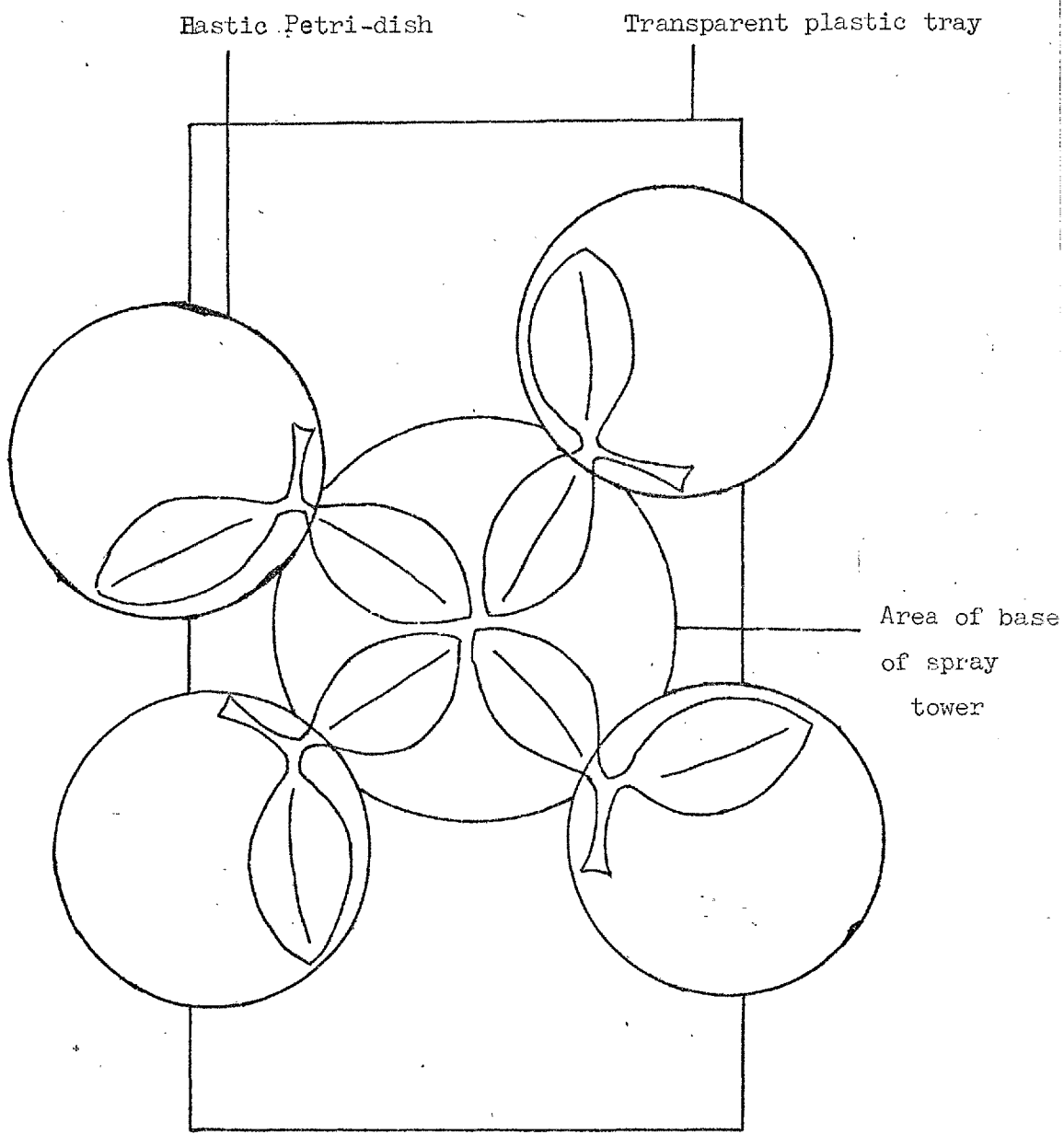
The precleaned slides were laid on clean absorbent paper with forceps. After spraying, the slides were put in boxes and used as for ordinary spore germination tests.

c) U.L.V. spraying equipment

i) Minimicron

This hand held sprayer developed by E.J. Balls of Micron Sprayers Ltd., Birmingham, is a development of the spinning disc principle of atomization and is illustrated in Plate 1. The atomizing head consists of a 9 cm diameter plastic disc with a serrated edge and turning at c. 7.000 rev/min on the engine shaft of a small two stroke petrol engine. The atomizer is designed to handle prepacked pesticide formulations and a standard one litre polythene container screws onto the atomizer head acting as a gravity feed reservoir. The oil contained in the reservoir flows down a tube onto the inside of the disc. Centrifugal force then releases the oil from the edge

Fig. 6. Four broad bean leaves arranged for spraying.



SERIAL No. 70/9

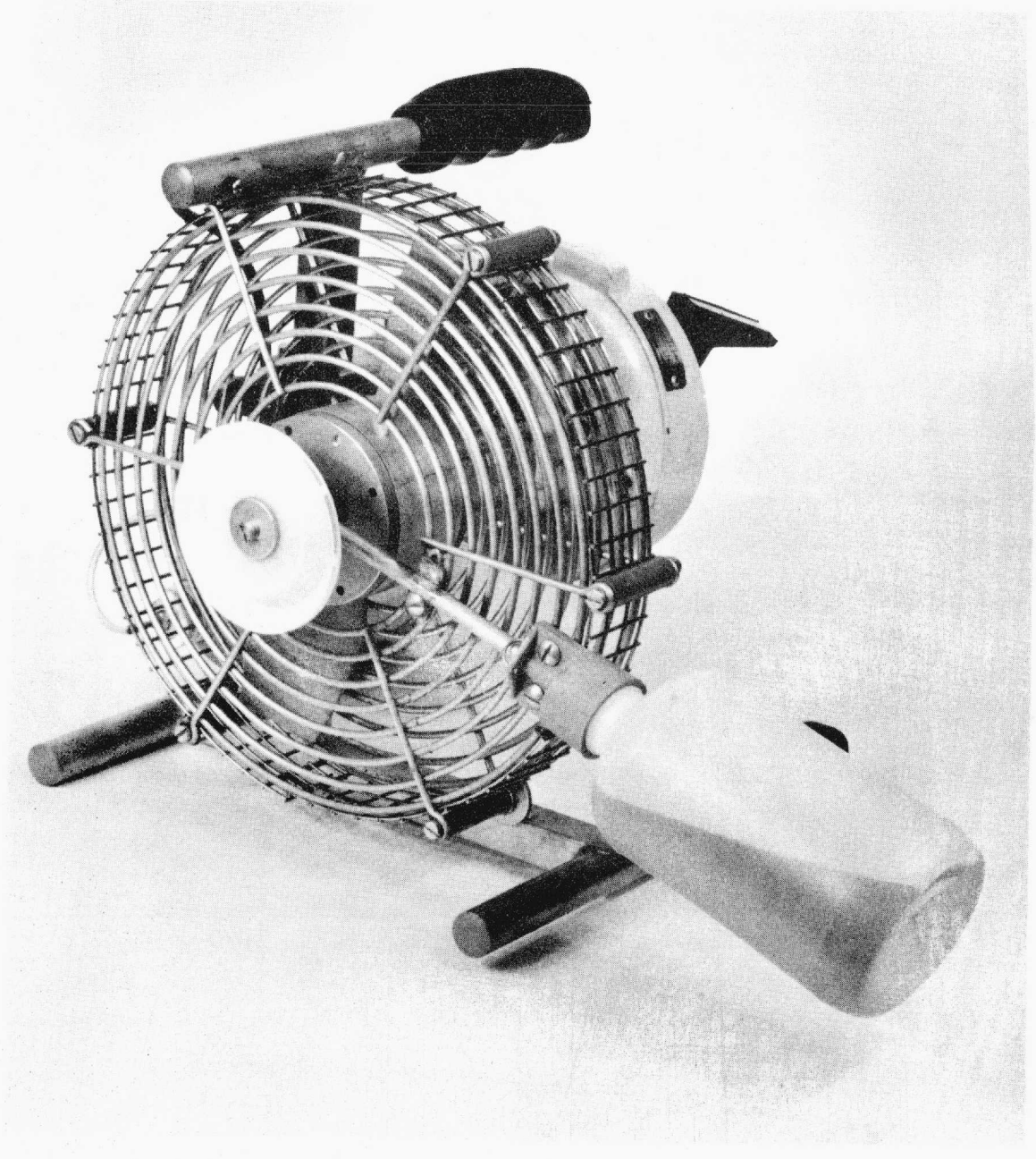


PLATE [redacted] I MINIMICRON SPRAYER

(Photograph by courtesy of the British Petroleum Company)

of the disc, the serration helping to break up the oil as it leaves so producing a very fine and even droplet spectrum. A fan situated in a cage immediately behind the disc and also turning on the engine shaft, directs the oil forward.

The Minimicron was used for spraying against downy mildew of vine and was held by the operator at knee height while walking up the rows of vines directing the spray obliquely at the plants so that the oil was deposited on the undersurface of the leaves. The machine was calibrated for each oil by measuring the oil lost from the reservoir after a 60 s spray.

The amount of oil deposited on leaves was determined by using weighed squares of aluminium foil attached to the leaves with paper clips. This method was used by Calpouzos et al. (1960) for determining the amount of oil deposited on banana leaves by commercial spraying methods. As the cutting of exact areas of foil was difficult and time consuming, areas were calculated from a calibration graph of area against weight.

The oil in the reservoir was measured before and after spraying and the spray rate calculated in litres per hectare. The aluminium foils were removed, folded to protect the sprayed side and immediately placed in clean Petri-dishes until they could be weighed. For gas oil which was very volatile (Appendix Ic), the foils were weighed within 30 min of spraying. For BP 85 and 150 neutral the foils were weighed on the same day as the spraying. At all times when handling the foils, the hands of the operator were kept free of oil.

ii) Micron ULVA

This is similar to the Minimicron but utilizes a lightweight motor drive powered from eight U-2 dry batteries. As with the Minimicron a

one litre polythene container screws onto the atomizer head acting as a gravity feed reservoir. The tube holding the batteries functions as a carrying handle and the sprayer is normally carried over the shoulder so that the operator walks away from the issuing spray. As there is no fan behind the atomizer head the makers, Micron Sprayers Ltd , recommend that use should be made of the prevailing wind to drift the spray over a swath of five or more metres.

The Micron ULVA was used in tests against vine downy mildew on isolated leaves. Instead of walking with the sprayer carried over the shoulder, the undersurface of the leaf was held up in one hand and the Micron ULVA held so that a very fine deposit of oil, barely noticeable to the naked eye, landed on the leaf surface.

The Micron ULVA was also used in the laboratory for spraying whole plants. It was set up inside the polythene tent used for the Potter tower so that the atomizing head was held horizontal when not in use the reservoir flask was below and the head above. On spraying the motor was switched on and the sprayer rotated on the axis of the battery tube so that the reservoir container was uppermost delivering oil by gravity to the atomizing head below. Deposits were varied by varying the spray time, the atomized oil drifting down by gravity onto plants arranged in the bottom of the tent. Initial calibration results using weighed circles of aluminium foil placed in the bottom of the tent showed that this method gave a reasonably acceptable distribution pattern.

When spraying the experimental oil fractions, the polythene container was removed and a small glass reservoir attached to the feed pipe of the atomizer head by means of a short piece of polythene tubing. After the motor was started, the oil was pipetted into the reservoir which was allowed to empty after which the motor was kept switched on for a standard

time to ensure complete discharge. The deposit for each spray was determined by placing weighed circles of aluminium foil among the plants and reweighing.

E. PHYTOTOXICITY ASSESSMENTS

Most observations were made on leaf discs which although useful for comparing oil fractions did not give a true indication of the phytotoxicity of any specific oil. Whole plants were therefore used for final tests of oil fractions which gave good disease control and low phytotoxicity on leaf discs. BP 85 which was a potential carrier oil for the more active oil fractions was tested more thoroughly on cucumber plants.

1. Leaf Discs

a) Vine. Necrosis and oil soaking

On vine leaf discs the appearance of necrotic areas varied with the type of oil used.

With the more phytotoxic oils of low boiling point these were relatively large and light brown to fawn in colour. The percentage area of leaf discs affected was estimated using the Horsfall-Barratt Grading System and standard area diagrams illustrated in Fig.2 .

Damage caused by higher boiling point oils was in the form of oil soaked areas and reddish brown necrotic spots both of which could be counted. Account was taken of the size of large areas and spots by taking the size of the smaller ones as standard and giving counts to the larger ones as appropriate. Results were expressed as the number of flecks/disc.

b) Cucumber Oil soaking and senescence (i)

Phytotoxicity was roughly graded in early experiments on a scale from 0 to 6 as follows:-

0 = no effect; discs light green in colour
1 = discs light green to yellow; no oil soaking
2 = discs yellow; no oil soaking
3 = discs yellow; light oil soaking
4 = discs yellow; heavy oil soaking
5 = discs partly decolourized; heavy oil soaking
6 = discs completely decolourized and oil soaked.

In later experiments oil soaking was estimated in terms of the percentage area of leaf disc affected using the Horsfall-Barratt Grading System (Horsfall and Barratt, 1945) and standard area diagrams illustrated in Fig. 2 . Leaf disc senescence was estimated by the chlorophyll assay method described below.

ii) Chlorophyll assay

The change in chlorophyll content of both uninfected and infected oil sprayed leaf discs was taken as a measure of direct oil phytotoxicity and of the combined effect of oil treatment and disease. The latter value would thus account for both the efficacy of the oil in controlling the disease and any interaction of oil and disease on the discs as well as direct phytotoxicity.

Leaf discs were decolourized separately in closed bijou bottles containing 3 ml of 70% ethanol. Chlorophyll was measured in terms of the optical density (D) at 665 mμ of the ethanolic extract using a Beckman DB spectrophotometer.

2. Whole Plants

a) Vine Necrosis

Necrosis was graded on an arbitary scale of 0 to 8 i.e. no damage to coalescence of damaged areas.

b) Cucumber

i) Necrosis

Necrotic areas on attached leaves were assessed by placing over

the leaf a card with a 20 mm diameter hole cut in it. The area of damage in the area cut off by the circle was estimated using the Horsfall-Barratt Grading System as for leaf discs. Ten representative areas were graded for each half leaf.

ii) Chlorophyll assay

Ten 10 mm leaf discs were cut from each half leaf and decolourized in 30 ml of 70% ethanol and the chlorophyll estimated as described for leaf disc assays.

iii) Membrane damage

This was measured in terms of ion leakage from oil treated leaves as detected by changes in electrical conductance of water containing treated leaf tissue. The technique was similar to that used by Goodman (1968) for measuring the effect of bacteria and chemicals on cell permeability. A Chandos A.21 linear conductivity meter obtained from Chandos Products (Scientific) Ltd., New Mills, Stockport, Derbys., was used in all experiments, conductance being measured in micro mho's.

Eight 17 mm leaf discs were cut from each replicate leaf or half leaf. These were transfered with a glass spatula to a clean glass tube (2.5 x 7.5 cm) containing 25 ml distilled water which had been left overnight to check the cleanliness of the tube. The discs were held under the water at the bottom of the tube with a glass ring whose diameter was just less than that of the discs. This left sufficient room for the electrodes of the conductivity meter to be placed in the water above the discs. Before measuring, the water and discs were agitated by removing the glass ring and allowing the discs to float to the surface. This was repeated, the ring replaced, and the electrodes placed in the tube and the conductance of the water measured. This was

repeated at intervals up to 48 h and the whole experiment carried out in a water bath at 25°C. Four replicates were used for each treatment, one half leaf being treated with oil and the other used as a control.

iv) Plant growth

The effect of oil on plant growth was determined by measuring internode length over a period of 20 d. Six replicate plants were used for each treatment.

EXPERIMENTAL SECTION I

THE USE OF OILS TO CONTROL VINE DOWNY MILDEW

INTRODUCTION

A light mineral oil (Esso Process 95) when misted alone on grapes was reported by Castellani and Matta (1962) to be effective in controlling downy mildew caused by Plasmopara viticola. The oil, which was highly refined with a high U.R. index was not fungitoxic to the fungus on leaves or to cultures of Botrytis cinerea or Gloeosporium musarum but penetrated through the stomata into the leaves. The mode of action of the oil on the pathogen or host was not reported. Skellern (1968) found, however, that on leaf discs the presumably similar mineral oil BP85 was not only ineffective in control, but also significantly increased sporulation. She also found that exudates from oil sprayed leaf discs were much less inhibitory to in vitro germination of sporangia than were exudates from unsprayed discs and suggested this as a reason for the increased disease on oil sprayed discs.

The present work attempts to clarify the apparent contradiction between the above reports by studying the action of different oils and oil fractions and also by comparing effects on leaf discs in the laboratory, on whole plants, and on attached leaves during field trials in France.

RESULTS

A. The Determination of Conditions necessary for Testing Oils against Vine Downy Mildew on Leaf Discs

To comply with standard procedures for evaluating fungicides (American Phytopathological Society, 1943), the effect of various factors on inoculum production and disease development were assessed first.

1. Comparison of methods of spore collection

Pegg and Mence (1970) found that the viability of sporangia of Peronospora viciae depended on the collection method used. When washed off in water germination was between 20 and 60%, whereas when shaken off dry and then suspended in water, germination was reduced to between 11 and 19%. Skellern (1968) using the dry suction method of collection described previously found that the viability of sporangia of Plasmopara viticola, in situ on vine leaves, was maximal in the early hours of the morning but declined as the day progressed and fell to between 5 and 15% if not collected until the following day. As the two fungi are from the same family it was thought that the effect of water conditions described above for P. viciae might be similar for P. viticola. Thus the effect of collection method, humidity and collection time were tested as described below.

a) The effect of humidity, collection method and collection time on the viability of sporangia

Six plants with heavily sporulating infections of vine downy mildew were produced as described previously. Three plants were kept in humid conditions over water in polythene bags for 24h while the other three were left uncovered in the same growth cabinet. At the beginning of the experiment c. $^{1}/_{3}$ of the sporulating leaf area of each of the six plants was harvested. Sporangia on one half of this area were washed off in distilled water and those on the other half were collected by suction. Suspensions were adjusted to 4×10^{5} sporangia/ml, and used to test the viability of the sporangia measured in terms of % germination on glass slides. Immediately after use, the suspensions were kept at c. 5°C for 8h and their germination tested again after checking to see if any germination had

occurred in the interval. At this time and 16h later the two collection methods were used again for the same purpose. Results are shown in Table 5.

Table 5 Germination of Sporangia of P.viticola as affected by collection method, collection time and humidity

Collection time (h)	Collection method	Culture conditions High r.h.	Low r.h.	8h Storage at c. 5°C
0	Washing	88.8	87.9	88.5
(11.30 h)	Suction	83.8	83.0	82.3
8	Washing	90.3	88.9	—
(19.30 h)	Suction	83.9	55.3	—
24	Washing	87.9	43.7	—
(11.30 h)	Suction	80.3	10.2	—

Discussion

There was a significant difference, between the germination of sporangia collected by the different methods, those collected by washing giving consistenly higher values, than those collected by suction. This is in agreement with similar results obtained by Pegg and Mence (1970) for Peronospora viciae. The difference is much more marked for sporangia which had been kept in situ on leaves at the lower humidity and would account for the results of Skellern (1968) assuming in that case that humidity was not maintained at a high level after the induction of sporangia. This suggestion is similar to that put forward by Weille (1963), that conidia of fungi belonging to the Peronosporales, when mature and attached to

conidiophores react differently to low relative humidity than those detached when mature. He suggested that free conidia were much less dependent on air relative humidity than still attached conidia, these presumably losing water *via* conidiophores to the leaf under low humidity conditions. Assuming this to be correct, the present results suggest that on detachment, conidia which have been kept under low humidity conditions for 8 h or more are more sensitive to damage or dry conditions of collection than are conidia detached after having been kept *in situ* under conditions of high humidity.

Up to 8h low humidity did not seem to adversely affect sporangia *in situ* if they were collected by washing. After 24h, however, even when washed off, germination was reduced by about 50%. Presumably in this case, many of the sporangia had irreversibly lost so much water as to prevent germination.

The storage of sporangia in suspension for 8h at *c.* 5°C was successful in temporarily preventing germination without adverse affecting viability as indicated by germination equal to that obtained before storage.

b) *The effect of collection method on the concentration, viability and state of sporangia recovered from old cultures*

The above experiment showed that the germination of sporangia kept *in situ* in humid conditions remained high up to 24h. In this experiment the two collection methods were tested using 5 d sporulating leaves from which two crops of sporangia had already been removed by suction. The third crop harvested in this experiment was 3 d old. In order to test the efficiency of the two methods of collection, 17 mm leaf discs were cut from sporulating areas of the leaves of three plants. The two methods were replicated on each leaf and the same number of discs used for each method.

Table 6 The concentration and state of sporangia recovered from old cultures of P.viticola (x 10^{-5} sporangia /ml)

State of sporangia	Collection method	
	Washing	Suction
Totally disrupted, granular or non-granular	7.5	3.8
Intact and non granular only	4.0	3.8

Table 7 The germination of sporangia recovered from old cultures of P.viticola

Collection method	Washing	Suction
Percentage germination	30.4	60.4
Percentage germination corrected for disrupted and granular sporangia	57.2	—

Discussion

The results in Table 6 show that as expected the washing method removes more sporangia than the suction method. Whereas, however, the suction method only removes intact, non-granular sporangia, the washing method also removes disrupted and granular sporangia.

As shown in Table 7, the percentage germination obtained with the suspension washed off the leaves was only 50% of that obtained by suction. When, however, account was taken of the number of disrupted and granular sporangia, there was no significant difference in germination between the two methods. It is likely that the granular sporangia described here are the same as those described by Gregory (1912) which he found did not germinate.

2. The effect of leaf age on sporulation

Skellern (1968) found that the thin pale green and shiny leaves numbered 3 to 7 back from the tip of rodstocks supported the highest sporulation. Older leaves only produced isolated patches of sporangia and younger leaves gave a thin sporophore weft. As vine seedlings were used in most experiments instead of rootstocks it was necessary to re-determine the effect of leaf age.

Leaf discs were cut from leaves of different ages from both young (ten to twelve nodes with about six to seven leaves) and older seedlings. They were numbered sequentially, with the first unfurled leaf number one. With young seedlings this was too small to use as were the first three on older seedlings. The discs were floated on water inoculated and incubated as described previously. Sporulation was estimated both visually and by determining the number of sporangia produced per disc.

Table 8 Sporangial production on leaf discs from young vine seedlings

Leaf number	Average sporulation grade	Sporulation ($\times 10^{-4}$ sporangia/disc)
2	2.3	15.6
3	1.3	1.9
4	0.7	0.3
5	0.4	0.04

Table 9 Sporangial production on leaf discs from old seedlings

Leaf number	Average sporulation grade	Sporulation ($\times 10^{-4}$ sporangia/disc)
4	2.8	17.3
5	2.7	16.5
6	2.5	14.0
7	2.1	9.1
9	1.8	5.7

Discussion

The main effect of leaf age was to limit the area of sporulation. On young discs this was well spread outside the area of the original inoculation drop whereas on older discs sporulation was almost restricted to this area and was less dense than on younger discs. Table 8 shows that with young seedlings sporulation fell off very quickly with leaf age indicating the necessity to use only the first few leaves. Table 9 shows that with older seedlings sporulation fell off much more gradually thus giving many more usable leaves than the younger seedlings.

Both tables show a reasonably close relation between the visual estimate and actual sporulation. A similar, non-quantitative relation was found by Malcolmson (1969) between a visual grading system and actual sporulation of Phytophthora infestans on potato leaflets.

3. The effect of the length of the wet period on infection

Royle (1970) found that the length of the period of wetness from inoculation to drying of the leaf surface was important in determining the

proportion of leaf area of hop infected by downy mildew caused by Pseudoperonospor humuli. To determine the importance of the wet period for infection by Plasmopara viticola, vine leaf discs were inoculated and the drops removed using a pipette and the disc surfaces dried in a stream of air from a fan at 2,4,6,12 and 24h after inoculation. Sporulation was estimated visually as indicated in Table 10.

Table 10 The duration of wetness required for leaf disc infection (numbers of discs)

Wet	Sporulation density					
period (h)	Heavy	Moderate	Light	Light/sparse	Sparse	Nil
2	0	0	0	1	2	15
4	0	0	0	1	6	11
6	0	0	0	3	8	7
12	0	0	1	4	5	8
24	0	7	4	3	2	2

Discussion

The results suggest that for maximum sporulation, at least 24h wetness is required. This was therefore used in all further experiments.

4. The effect of light on infection

D.J. Royle (personal communication 1970) found that light was necessary for infection of hop by Pseudoperonospora humuli. The effect of light on infection of vine by Plasmopara viticola has not been determined and was therefore investigated to find the most suitable conditions for later experiments on the effect of oils on the disease.

In the first experiment leaf discs were cut at midday from leaves which had been kept on plants in the light. Discs were inoculated and placed in a closed light proof tin at 0, 3, 6 and 11h after inoculation. Sporulation was visually graded at 5 d after inoculation with the 11h light treatment graded again at 10d. In a second experiment sporulation was estimated at 5, 6 and 11 d after post inoculation light treatments of 0, 2 and 7h . Results are shown in Table 11.

Table 11 The sporulation of leaf discs as affected by light after inoculation (Average sporulation grades)

1st EXPERIMENT

Light	Estimation time (days after inoculation)	
period (h)	5	10
0	3.1	*
3	3.0	*
6	2.2	*
11	0.6	2.2

2nd EXPERIMENT

Light	Estimation time (days after inoculation		
period (h)	5	6	11
0	0.83	2.2	2.9
2	0.63	2.5	3.2
7	0.13	1.3	2.3

* = not estimated.

Discussion

In the first experiment the 6 and 11 h light treatments significantly reduced sporulation at 5 d. At 10 d the sporulation on the 11 h treated discs had increased. Thus, to determine whether the light delayed or reduced sporulation, a second experiment was carried out and estimations made at 5, 6 and 11 d after inoculation. As shown in Table 11, at 5 d, light treatments again reduced sporulation, and although this had increased by 11 d, sporulation on the 7 h light treatment was still significantly less than the 0 and 2 h treatments. Thus, future experiments were carried out in the evening with an initial period of 2 to 3 h before the lights in the growth cabinet went cut.

B. A Comparison of Oil Types for Control of Vine Downy Mildew on Leaf Discs

1. Wide distillation range oils

a) BP85

To investigate the results of Skellern (1968), leaf discs were sprayed with BP85 at levels of 0.15, 0.30, 0.60 and 0.90 ml/ giving deposits of 4.2, 11.0, 24.4 and 35.4 mg/100 cm^2 using the pipette method of spraying with the Potter tower (as described previously). These rates were equivalent to commercial spray applications of 5.5, 13.7, 30.0 and 43.5 l/ha (0.5, 1.2, 2.7 and 3.9 gal/ac) using the Minimicron during field trials in France in 1969. Discs were inoculated giving the results shown in Table 12. Sporulation was visually graded as before.

Table 12 The effect of BP85 on vine downy mildew

Oil Level (ml)	Sporulation grade
0	2.5
0.15	2.9
0.30	2.9
0.60	2.9
0.90	3.0

Discussion

The results confirm those of Skellern (1968), BP85 having no obvious effect on the disease. No significant increase in sporulation was detectable on oil sprayed discs, though the assessment method used here would probably not show up small differences between heavily sporulating discs as would the sporangial counts used in later experiments and by Skellern (1968).

b) A comparison of BP85 with other oils

To determine whether the control of vine downy mildew with an Esso mineral oil reported by Castellani and Matta (1962) was due to the type of oil used, BP85 was compared with the other oils, gas oil and 150 neutral. Both gas oil and BP85 were passed through a silica-gel column to extract the (A + H) components and both the original whole oil (w.o) and the (N+P) component were tested. As Skellern (1968) found that when BP85 was exposed to sunlight for a few weeks, it became toxic to the sporangia of Plasmopara viticola, a sample of oil was similarly exposed to sunlight for a few months and also included in the experiment. Discs were sprayed with the oils at a level of 0.15 ml and inoculated as before giving the results shown in Table 13. Sporulation was estimated as in the previous experiment.

Table 13 The effect of gas oil, BP85 and 150 neutral on vine downy mildew

Oil type		Sporulation grade
Control		2.3
Gas (W.O.)		1.8
Oil (N + P)		2.3
BP85	Light	1.9
	(W.O.)	2.3
	(N + P)	2.2
150 neutral		2.3

(W.O.) = Whole oil containing both (N+P) and(A+H) components

(N + P) = Whole oil from which the (A + H) component has been removed

LIGHT = Oil exposed to sunlight

Discussion

A significant reduction in sporulation was obtained using gas oil containing the (A + H) component but none for the same oil without this component. Thus the (A + H) component would seem to be responsible for the reduction in sporulation. Neither the normal BP85 or 150 neutral had any effect on the disease, though the sample of BP85 exposed to sunlight reduced sporulation to a similar extent as gas oil (whole oil). However, in neither case was control at a commercially acceptable level.

2. Experimental oil fractions

a) Saturated fractions

As the wide distillation range oils gave no mildew control, it was thought that if mineral oil was at all effective, the active ingredient might be contained in a very narrow distillation range. Thus four fractions (No's 19, 21, 22, 23) were tested covering the range 297°C to 374°C, each one having a narrow distillation range of from 15°C to 17°C (Table 4). Oils were applied at the 0.15 ml level, leaves were inoculated and sporulation graded as in the last experiment. Results are shown in Table 14.

Table 14 The effect of saturated oil fractions on vine downy mildew

Fraction number	Sporulation grade
19	3.5
21	2.6
22	2.9
23	3.1
Control	3.5

Discussion

Fraction 21 was the only oil to give any reduction in sporulation, though this was only slight and not at a commercially acceptable level. As gas oil containing an (A + H) component was shown to reduce sporulation it was decided to compare saturated and unsaturated components as described below.

b) A comparison of saturated and unsaturated fraction components

The (A + H) and (N + P) components of fraction 22 were compared together with BP85 at the 0.15 ml level. Discs were inoculated and sporulation estimated in terms of grades and the average number of sporangia per disc.

Table 15 The effect of fraction 22 components on vine downy mildew

Treatment		Sporulation grade	% Control based on grades	Sporangia/disc (x 10^{-3})	% Control based on counts
Control		1.7	—	29.8	—
Fraction 22	(A+H)	0.2	88.2	3.3	88.9
	(N+P)	1.5	11.8	21.7	27.1
BP85		1.3	23.5	15.5	37.6

Discussion

As shown in Table 15 the (A+H) component of fraction 22 was much more active than the (N+P) component giving more than 88% control and showing possible commercial potential. The calculation of percentage control base on grades and counts of sporangia showed very good agreement though at lower values agreement was not so close. The degree of control obtained with the

(N+P) component was similar to the previous experiment although BP85 gave an even greater reduction in sporulation. This result was unexpected as in previous experiments BP85 had been shown to increase sporulation even at high levels. However, during field experiments in France BP85 was shown to control the disease to a similar extent. Thus, one can only conclude that under certain unspecified conditions BP85 is able to produce a slight reduction in the sporulation of vine downy mildew.

It was, however, clear that the unsaturated component of oil fractions might provide commercially acceptable control of the disease.

C. The Effect of the Unsaturated Components of Oil Fractions on Disease and Phytotoxicity using Leaf Discs

The unsaturated components of oil fractions consist of aromatic and heterocyclic hydrocarbons. Havis (1950) showed that aromatics were more phytotoxic than any other group and were followed by naphthenes olefins and straight chain paraffins in order of toxicity. Boiling point was shown to influence toxicity independently of hydrocarbon series, the range of greatest toxicity being between 138°C and 266°C. Below this range toxicity was low and above chronic injury was encountered. Thus the safeness of oil applications to plant leaves is related to the unsaturated components, particularly the aromatic content of oil (Chapman and Pearce, 1947).

In view of these facts it was very important to carry out studies on phytotoxicity along with those on fungitoxicity. Initial experiments used selected fractions in the range from 210°C to 392°C. In later experiments the range was narrowed to a few fractions which showed low phytotoxicity and promising fungitoxic activity.

1. Comparison of (A + H) Components

a) Preliminary comparisons

EXPERIMENT 1

In this experiment oils were applied at the 0.1 ml level as before to leaf discs cut from leaves of vine cv. "Black Hamburg" and floated on water in closed Petri-dishes. Six discs were used for each treatment and inoculations were carried out as before. Sporulation was visually estimated as indicated in Table 16.

Table 16 The effect of (A+H) components on vine downy mildew

Fraction number	Sporulation density (total of 6 discs/treatment)			
	Heavy	Moderate	Light	Sparse
13	0	3	3	0
16	0	1	5	0
19	0	0	3	3
22	0	3	3	0
24	5	1	0	0
Control	6	0	0	0

Discussion

Fraction 19 showed most activity, which fell off with lower boiling fractions and even more markedly for higher boiling fractions. This suggested that molecular size may havebeen the determining factor, higher boiling fractions containing molecules too large to be effective and lower boiling fractions being so volatile that they did not remain long enough on the leaf. The results of volatility tests described in Appendix 1 C suggests that fractions 13 and 16 would have evaporated almost immediately.

EXPERIMENT 11

In this experiment "Black Hamburg" was again used and oils sprayed as before at levels of 0.1, 0.2, 0.3 and 0.6 ml on leaf discs which were floated on water in closed Petri-dishes as before. Discs from the same leaves were sprayed at levels of 0.1, 0.3, 0.6 ml and floated on water in open Petri-dishes to allow volatile oils to evaporate. Eight discs were used for each treatment and at 5 d sporulation was estimated visually as indicated in Table 17 and at 7 d using a scale from 0-5 as described previously, the results being given in Table 18. Phytotoxicity was estimated at 12 h and 7'd after spraying in terms of the percentage leaf disc area affected using the Horsfall-Barratt grading system as described previously, the results being given in Table 19.

Table 17 (see overleaf)

Table 17 The effect of oil fractions and oil level on the sporulation of vine downy mildew and on phytotoxicity at 5 d

Fraction number	Oil level (ml)	Sporulation density (8 discs)			Phytotoxicity comments
		Moderate	Light	Sparse	
13	0.1	0	0	2	1 disc heavy
	0.2	0	0	0	5 discs heavy
	0.3	0	0	0	8 discs heavy(100%)
	0.6	0	0	0	8 discs heavy(100%)
16	0.1	0	0	0	2 discs heavy
	0.2	0	0	0	8 discs heavy(100%)
	0.3	0	0	0	8 discs heavy(100%)
	0.6	0	0	0	8 discs heavy(100%)
19	0.1	0	0	3	flecking
	0.2	0	0	1	gradually
	0.3	0	0	1	increases
	0.6	0	0	0	
22	0.1	3	2	3	Nil
	0.2	2	2	4	Nil
	0.3	1	3	4	Nil
	0.6	0	1	7	flecking in innoculation area
24	0.1	5	2	1	Nil
	0.2	5	1	2	Nil
	0.3	3	2	3	Nil
	0.6	1	1	6	Nil
Control	—	5	2	1	

Table 18 The effect of oil fractions and oil level on the sporulation of vine downy mildew at 7 d

Fraction	Petri-dish closed				Petri-dish open		
	Oil level (ml)				Oil level (ml)		
Number	0.1	0.2	0.3	0.6	0.1	0.3	0.6
13	2.3	0	0	0	Not	0.8	0
16	0	0	0	0		0	0
19	0.5	0.1	0.1		estimated	0	0
22	3.3	2.5	2.8	2.8	Control =	3.6	
24	3.5	3.6	3.5	2.3			

Discussion

In this experiment the heavy phytotoxicity of fractions 13 and 16 at 5 d was particularly noticeable. These fractions had boiling ranges of 210°C to 224°C and 252°C to 264°C and according to Havis (1950) would also be expected to be phytotoxic. It is possible that the results obtained here were due to either a) the use of leaf discs, oil penetrating the cut edges or b) the enclosed conditions in the petri-dishes preventing the oil from evaporating. As shown in Experiment 1, fractions 22 and 24 were much less effective than the lower boiling fractions 19, 16 and 13. The phytotoxicity of these latter two precluded their use in any further experiments.

On closer examination of the flecking produced by fraction 19, this was seen to be in the form of browning in or below the epidermal layer. Inoculated discs treated with this fraction showed no visible sporulation when observed through a dissecting microscope but when observed at a magnification of x 100, very short sporangiophores could occasionally be

seen together with much browning.

Sporulation at 7 d given in Table 18 showed that at the 0.1 ml level fraction 13 was much less effective than any other levels of this fraction or than any levels of fractions 16 and 19. At the higher levels of 0.3 ml and 0.6 ml, disease control on discs in open dishes was no different for fractions 13, 16 and 19 than for discs in closed dishes.

Table 19 The percentage leaf disc area damaged at 12 h and 7 d in closed or open Petri-dishes

12 h assessment (average % damage/disc)

Fraction Number	Petri-dish closed Oil level (ml)				Petri-dish open Oil level (ml)		
	0.1	0.2	0.3	0.6	0.1	0.3	0.6
13	3.1	35.0	> 99	100	0	0	4.1
16	< 1	73.0	95.8	> 99	0	0	93.6
19	0	0	0	0	0	0	0
22	0	0	0	0			
24	0	0	0	0			

7 h assessment (average % damage/disc)

Fraction Number	Petri-dish closed Oil level (ml)				Petri-dish open Oil level (ml)		
	0.1	0.2	0.3	0.6	0.1	0.3	0.6
13	6.6	94.0	100	100	0	0	87.2
16	19.0	100	100	100	0	24.6	100
19	0	1.7	3.8	8.8	0	91.0	100
22	0	0	0	0			
24	0	0	0	4.5			

Phytotoxicity at 12 h and 7 d was clearly different in closed and open Petri-dishes as shown in Table 19;at 12 h both fractions 13 and 16 were much more phytotoxic in closed Petri-dishes than in open ones. At the 0.3 ml level fraction 13 caused more than 99% of the surface of leaf discs in closed dishes to be damaged, whereas no damage occurred on discs in open dishes. For fraction 16 the figure of 95.8% for closed dishes applied similarly. Even at 0.6 ml fraction 13 only caused 4.1 % damage in open dishes whereas fraction 16 caused 93.6 % damage at the same level.

At 7 d phytotoxicity had increased considerably with even fraction 19 at the 0.3 ml level causing 91 % damage on discs in open dishes. Damage was, however, still negligible for fractions 22 and 24 at any level. The results suggested that fraction 19 was doubtful for commercial development even though no damage was evident at the 0.1 ml level, there being too little latitude for error in application.

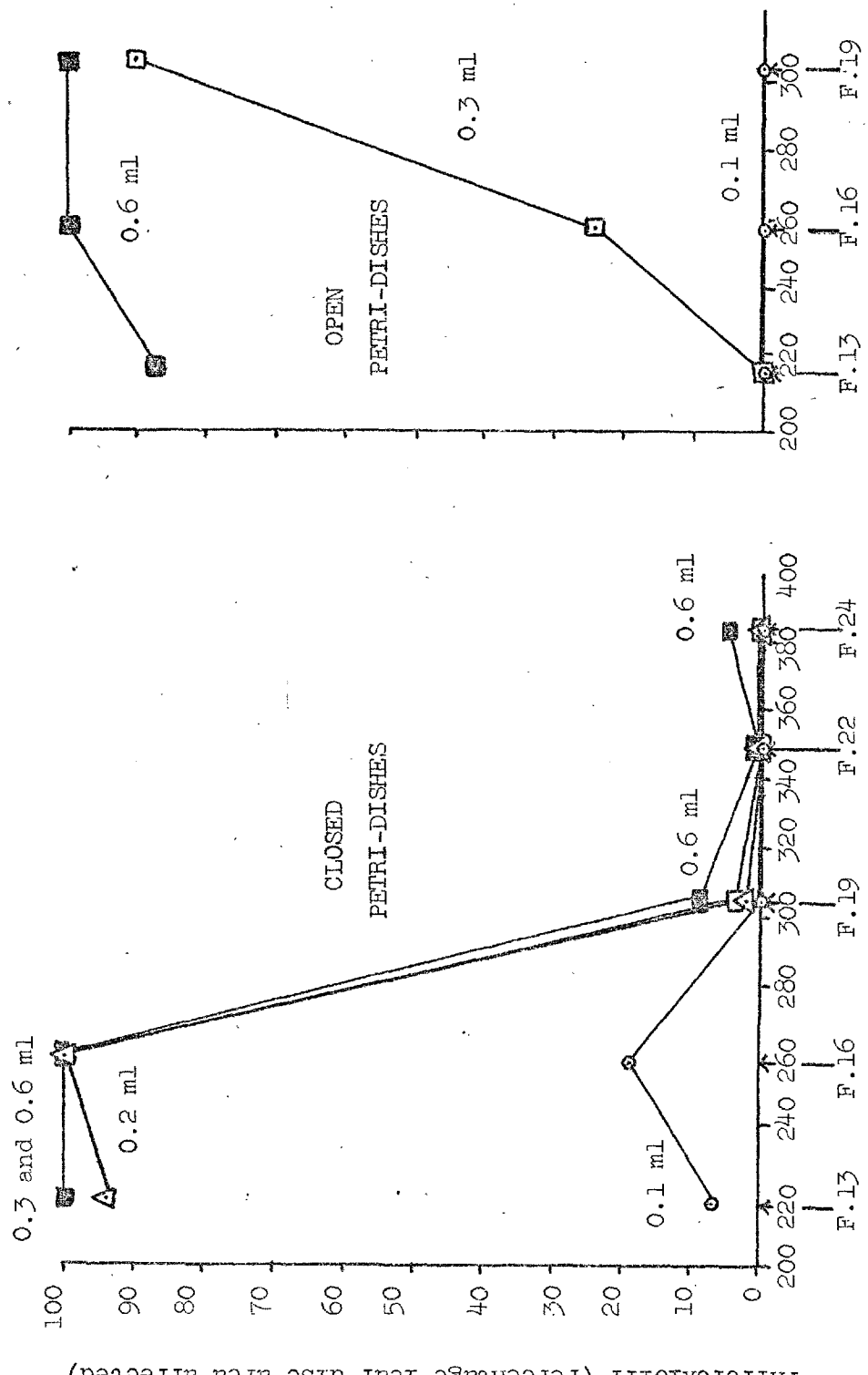
Plotted graphically as in Fig. 7 the effect of maintaining the discs in open or closed dishes on the phytotoxicity of the oils can be clearly seen. Furthermore, the effect of these conditions on fractions 13 and 16 is opposite to that on fraction 19. Whereas open dishes reduce the phytotoxicity of the former two as compared with closed dishes , for fraction 19 phytotoxicity is dramatically increased from, for example, 3.8 % at 0.3 ml in closed dishes to 91.0 % at the same level in open dishes.

Thus in the following experiment, the effect of open and closed Petri-dish conditions was tested on the phytotoxicity of oils between fraction 19 and fraction 24.

b) The effect of leaf age and water stress on phytotoxicity

As it is generally accepted that under conditions of high water stress plants are much more susceptible to oil damage than under low water stress conditions (Personal Communication, British Petroleum Co. Ltd.) it was thought likely that this would explain the results using the open and closed

Fig. 7. The effect of open or closed Petri-dish conditions on (A + H) Component Phytotoxicity



Petri-dishes of the last experiment. Thus in closed Petri-dish conditions, the resulting high humidity and low water stress would reduce phytotoxicity caused by fraction 19 under open Petri-dish, and therefore low humidity--relatively high water stress conditions. To test this idea more fully, discs were cut from leaf 6 of vine cv. "Black Hamburg", numbering being sequential with the first unfurled leaf number one, sprayed with fractions 19, 22 and 24 at levels of 0.1 ml and 0.2 ml as before, and treated in one of three ways viz. either 1) sprayed with distilled water in the Potter tower and maintained in closed Petri-dishes or

2) not sprayed with water but kept in closed dishes or

3) not sprayed and kept in open Petri-dishes.

The effect of leaf age was also tested in the same experiment using discs cut from leaves 4,5,6,7,9, and 11 and spraying them with distilled water in the Potter tower to simulate natural infection conditions that would be encountered after rainfall.

Phytotoxicity was estimated 1, 8 and 14 d after oil spraying, necrotic spots and oil soaked areas being estimated separately and the results expressed in numbers per disc. Thus both the effects of water stress and leaf age on phytotoxicity were determined in terms of the two types of damage

Table 20 (see overleaf)

Table 20 The effect of leaf age on phytotoxicity (Total number of necrotic spots and oil soaked areas/ disc)

Estimation time d	Leaf number	Fraction 19 Oil level (ml) 0.1	Fraction 19 Oil level (ml) 0.2	Fraction 22 Oil level (ml) 0.1	Fraction 22 Oil level (ml) 0.2	Fraction 24 Oil level (ml) 0.1	Fraction 24 Oil level (ml) 0.2
1	4	0.1	0.8	0	0	0	0
	5	1.0	1.2	0	0	0	0
	6	1.7	2.6	0	0	0	0
	7	1.4	3.4	0	0	0	0
	9	1.0	2.2	0	0	0	0
	11	0.9	3.3	0	0	0	0
8	4	1.2	12.5	0.1	0.8	1.2	1.5
	5	2.5	10.3	0.5	0.8	3.0	1.0
	6	2.0	7.5	0.5	1.7	1.6	6.1
	7	4.0	7.0	0.6	0.7	5.4	9.5
	9	2.8	4.8	0.7	0.7	10.1	9.1
	11	3.0	7.2	0.7	0.1	4.7	11.5
14	4	2.4	12.5	0.2	1.8	1.2	10.5
	5	2.9	10.3	0.5	2.1	4.4	8.6
	6	2.9	7.5	0.8	6.2	3.4	43.8
	7	4.0	7.7	1.2	9.3	10.7	51.0
	9	2.8	5.9	2.4	12.1	16.9	52.9
	11	3.2	8.6	1.3	6.0	10.3	47.1

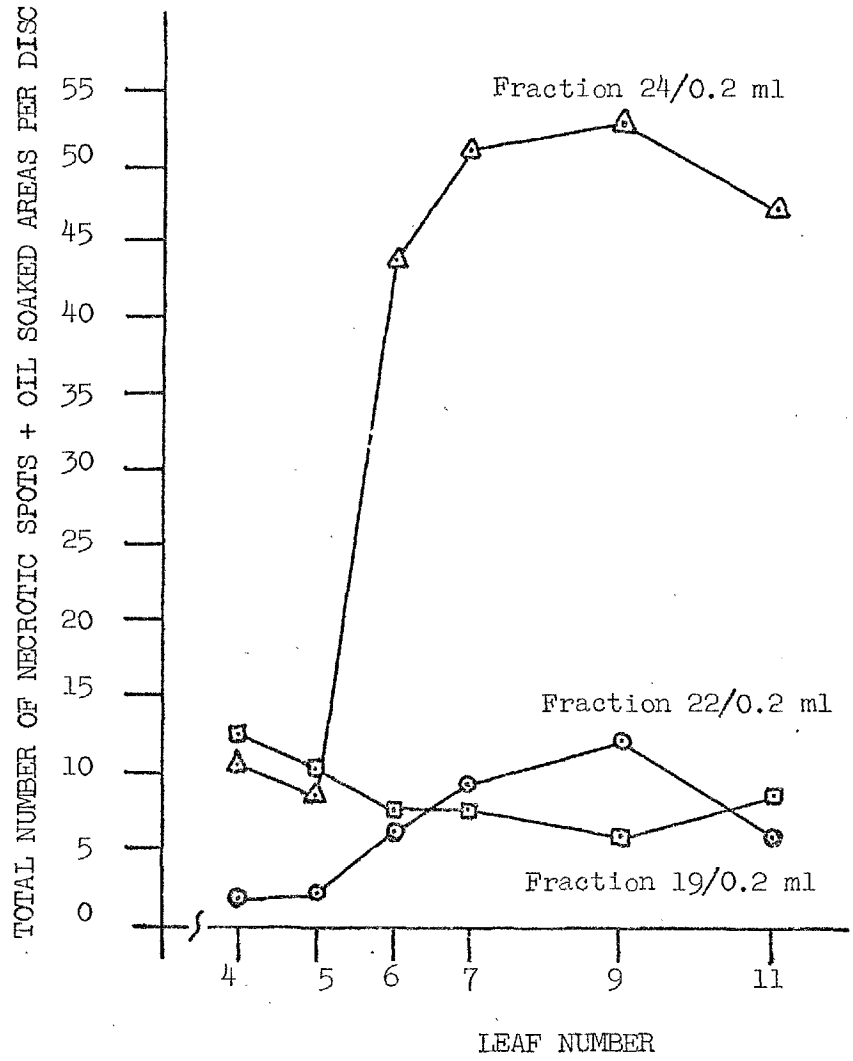
Table 21 The effect of leaf age on phytotoxicity type (Number of necrotic spots [N.S.], oil soaked areas [O.S.] or both [T]/disc)

		Fraction 19						Fraction 22					
Estimation		0.1 ml oil level			0.2 ml oil level			0.1 ml oil level			0.2ml oil level		
time	Leaf	Phytotoxicity			Phytotoxicity			Phytotoxicity			Phytotoxicity		
(d)	age	N.S.	O.S.	T.	N.S.	O.S.	T.	N.S.	O.S.	T.	N.S.	O.S.	T.
	4	1.0	0.2	1.2	12.5	0	12.5	0	0.1	0.1	0.2	0.6	0.8
	5	2.5	0.5	2.5	9.9	0.4	10.3	0	0.5	0.5	0.1	0.7	0.8
	6	1.0	1.0	2.9	5.6	1.9	7.5	0.1	0.4	0.5	0.7	1.0	1.7
8	7	2.7	1.3	4.0	6.2	0.8	7.0	0.4	0.2	0.6	0.7	0	0.7
	9	2.5	0.3	2.8	3.6	1.2	4.8	0.5	0.2	0.7	0.3	0.4	0.7
	11	1.6	1.4	3.0	4.3	2.9	7.2	0.3	0.4	0.7	0	0.1	0.1
	4	1.3	1.1	2.4	12.6	0.6	13.2	Phytotoxicity of fraction					
	5	2.1	0.8	2.9	10.0	1.0	11.0	22 only estimated in					
14	6	2.0	0.9	2.9	5.8	1.8	7.6	terms of total damage					
	7	2.8	1.2	4.0	6.6	1.1	7.7						
	9	2.4	0.4	2.8	4.3	1.6	5.9						
	11	1.9	1.3	3.2	4.5	4.1	8.6						

Discussion on the effect of leaf age on phytotoxicity

Table 20 shows particularly with fraction 22 at the 0.2 ml level and fraction 24 at both the 0.1 ml and 0.2 ml level that at 14 d the younger leaves 4 and 5 were more resistant to oil damage than older leaves. Total damage increased to a maximum on the mature leaves 6,7 and 9 but decreased again on leaf 11. With fraction 19, damage tended to be slightly greater on the younger leaves at the 0.2 ml level. Fig. 8 shows the line for

Fig. 8. The effect of leaf age on phytotoxicity at 14 days



fraction 19 being an almost mirror image of fraction 22. Table 20 also shows that fraction 19 caused phytotoxic symptoms earlier than either fraction 22 or 24. It also shows together with Fig.8 that fraction 24 was by far the most phytotoxic of the three fractions. Table 21 shows phytotoxicity in terms of necrotic spots or oil soaked areas. For fraction 24 at 8 d and fraction 24 and 22 at 14 d it was difficult to distinguish between the two types of phytotoxicity and thus only totals of both type were noted. The majority of damage caused by fraction 19 was in the form of necrotic spots rather than oil soaked areas particularly at the 0.2 ml level. The effect of water stress is shown in Tables 22 and 23.

Table 22 The effect of water conditions on phytotoxicity (Total number of necrotic spots and oil soaked areas/disc)

Assessment time (d)	Treatment conditions	Fraction 19 Oil level (ml)		Fraction 22 Oil level (ml)		Fraction 24 Oil level (ml)	
		0.1	0.2	0.1	0.2	0.1	0.2
1	*-W/open	4.5	11.7	0	0	0	0
	*-W/closed	2.0	5.7	0	0	0	0
	*+W/closed	1.7	2.6	0	0	0	0
8	*-W/open	5.0	17.6	1.4	2.8	8.6	21.8
	*-W/closed	3.7	9.4	0.3	0.9	3.3	11.0
	*+W/closed	2.9	7.5	0.5	1.7	1.6	6.1
14	*-W/open	5.8	17.4	1.6	–	11.8	69.5
	*-W/closed	5.0	9.9	0.4	–	6.8	35.6
	*+W/closed	2.8	7.1	0.8	–	3.4	43.8

* + W = discs sprayed with water; - W = discs unsprayed

Table 23 The effect of water conditions on phytotoxicity type (Number of necrotic spots, oil soaked areas or both)

Assessment time (d)	Treatment conditions	Fraction 19						Fraction 22					
		0.1ml oil level			0.2ml oil level			0.1ml oil level			0.2ml oil level		
		Phytotoxicity			Phytotoxicity			Phytotoxicity			Phytotoxicity		
		N.S.	O.S.	T.	N.S.	O.S.	T.	N.S.	O.S.	T.	N.S	O.S.	T.
8	-W/open	4.8	0.2	5.0	13.8	3.8	17.6	0.7	0.7	1.4	0.8	2.0	2.8
	-W/closed	2.4	1.3	3.7	6.1	3.3	9.4	0	0.3	0.3	0.2	0.7	0.9
	+W/closed	1.9	1.0	2.9	5.6	1.9	7.5	0.1	0.4	0.5	0.7	1.0	1.7
14	-W/open	4.8	1.0	5.8	13.8	4.6	18.4						
	-W/closed	2.9	2.1	5.0	7.0	3.3	10.3						
	+W/closed	1.9	1.0	2.9	5.6	2.0	7.6						

Discussion of the effect of water stress

The results in Table 23 clearly show the difference between open and closed Petri-dishes and again that fraction 22 is less phytotoxic than fraction 19 with fraction 24 causing by far the greatest damage. With fraction 19 at the 0.2 ml level discs sprayed with water and maintained in closed dishes showed the least phytotoxicity followed by discs in unsprayed closed dishes and finally, the highest phytotoxicity occurred on unsprayed discs in open dishes. Fraction 24 showed the same progression though at the 0.2 ml level phytotoxicity was so high and variation between discs so large that there was no significant difference between sprayed and unsprayed discs in closed dishes.

As shown in Table 23 phytotoxicity caused by fraction 19 which was mainly in the form of necrotic spots was clearly reduced by maintaining the discs in humid conditions. There was also a slight suggestion that phytotoxicity with fraction 22 might be more in the form of oil soaked areas. This was examined further as shown below.

c) The effect of temperature on the phytotoxicity of fraction 22

Discs were cut from leaves 3 and 5 of vine cv. "Black Hamburg" sprayed as before at the 0.2 ml level and floated on water in open Petri-dishes. The dishes were placed in germinating trays with the lids of the trays supported so that the dishes were covered leaving a gap so that the discs were not exposed to humid conditions. The trays were then placed in the following conditions:-

1. A cooled lighted growth cabinet at c. 14°C
11. A cooled lighted growth cabinet at c. 20°C
111. A non-cooled lighted growth cabinet at c.20°C - 35°C with the lights on during the night and off during the day to prevent the temperature rising too far.
1V. The college greenhouse giving somewhat wider temperature fluctuations than 111 of c.15°C - 40°C. Lighting was similar to that in growth cabinets.

Phytotoxicity was estimated in terms of the average number of necrotic spots or oil soaked areas per disc. Results are shown in Table 24.

Table 24 The effect of different environments on the phytotoxicity of Fraction 22

Assessment time (d)	Leaf number	Environment 1 N.S.	1 O.S.	11 N.S.	11 O.S.	111 N.S.	111 O.S.	1V N.S.	1V O.S.
	3	0	2.4	0	10.8	0	0.6	—	—
4	5	0	1.8	0.8	7.0	0.6	3.0	4.4	13.6
	3	1.8	10.6	0	15.6	0.6	0.6	—	—
8	5	0.4	15.5	0	18.0	0	3.2	9.0	20.2

Discussion

In this experiment it was very clear that fraction 22 phytotoxicity was mainly in the form of oil soaked areas. It is difficult to explain, however, why the conditions of environment 111 should cause the oil to be less phytotoxic than the others. It is possible that the relatively high temperatures of environment 111 may have caused the oil to evaporate at a sufficiently rapid rate as to prevent phytotoxicity. At the lower temperatures of environment 1 and 11 no significant evaporation would have occurred whereas at the higher temperatures of environment 1V, the effect might have been to reduce oil viscosity sufficiently enough to enable it to penetrate into the leaf tissue before it could evaporate.

Apart from environment 111 the results suggest that there is no effect of temperature on phytotoxicity. The literature is contradictory on this point (Havis, 1950; Dallyn and Sweet, 1951) and the experiment described here was not precise enough to justify any conclusions.

d) A comparison of fractions 21, 22 and 23 for disease control and phytotoxicity

Although fraction 22 was the least phytotoxic oil so far tested, it was still capable of causing considerable damage as shown in the last experiment. Thus the two fractions above and below fraction 22 were tested in an attempt to find a less phytotoxic oil whilst maintaining high fungitoxic activity.

Discs were cut from seedlings of cv. "Riesling" which was used in all further experiments. Oils were sprayed at the 0.1ml and 0.2ml levels and the discs inoculated and incubated in staining dishes (as before). Sporulation was estimated in terms of the number of sporangia per disc.

Phytotoxicity was studied in a second experiment using discs floating on water in open and closed Petri-dishes with necrotic spots and oil soaked areas counted separately as before. In this experiment oils were applied at the 0.15 ml level.

Table 25 The effect of fractions 21,22 and 23 on vine downy mildew

Fraction number	$x\ 10^{-3}$ Sporangia/disc		% Control*	
	Oil level (ml)		Oil level (ml)	
	0.1	0.2	0.1	0.2
21	4.7	2.5	85.1	92.3
22	7.3	5.0	77.7	84.6
23	14.6	5.7	55.1	82.6

* Control = 39.0 $x\ 10^{-3}$ Sporangia/disc

Table 26 The phytotoxicity of fractions 21, 22 and 23

Assessment time (d)	Fraction number	Petri-dish open			Petri-dish closed		
		Phytotoxicity type			Phytotoxicity type		
		N.S.	O.S.	T.	N.S.	O.S.	T.
4	21	0.1	0.1	0.2	0.2	0.7	0.9
	22	1.6	5.7	7.3	0.5	3.0	3.5
	23	0	0.1	0.1	0	0	0
13	21	0.1	0.4	0.5	1.0	4.5	5.5
	22	15.1	6.4	21.5	6.5	10.0	16.5
	23	0.4	1.1	1.5	0.2	1.1	1.3
23	21	0.1	0.4	0.5	1.0	6.1	6.5
	22	22.7	7.8	30.5	11.4	7.2	18.6
	23	0.6	1.3	1.9	1.4	10.4	11.8

Discussion

The results of the first experiment given in Table 25 show that disease control increased with decreasing oil fraction boiling point. Fraction 23 showed a much larger increase in control from the 0.1 ml to 0.2 ml oil level than either fraction 21 or 22. Thus fraction 23 was much more active at the higher level than would have been expected from its activity at the lower level. This suggests that its activity was not just due to a simple protective effect.

Fraction 21, as shown in Table 26 was much less phytotoxic than fraction 22 and although damage at 13 d was greater than for fraction 23, by 23 d fraction 23 showed more phytotoxicity than fraction 21. This is consistent with previous results where fraction 19 showed phytotoxicity at an earlier date than fraction

22, and is expected in that lower boiling point (and thus lower molecular weight and less viscous) hydrocarbons will take longer to penetrate and act within the tissue.

If the effect of using open or closed Petri-dishes is assumed to be one of low or high humidity, the effect of the latter on the phytotoxicity of the three oils is very clear. At 23 d the high humidity conditions increased the phytotoxicity of both fractions 21 and 23 mainly in the form of oil soaked areas whereas necrotic spot damage was not significantly affected. With fraction 22 the reverse was the case, high humidity conditions reducing phytotoxicity in the form of necrotic spots. This result contradicts that of previous experiments where fraction 22 phytotoxicity was mainly in the form of oil soaked areas. It was noticed, however, that during the experiment, many of the oil soaked areas on fraction 22 sprayed discs gradually turned brown and thus would at a later date be counted as necrotic spots. It is likely, therefore, that the lower molecular weight and less viscous fraction 22 caused damage initially in the form of oil soaked areas which then browned rapidly. This is confirmed by the 4 d results, damage for this fraction being mainly oil soaked areas. With the higher molecular weight and more viscous fraction 23 phytotoxic action would have progressed at a slower rate and thus at 23 d damage was mainly in the form of oil soaked areas which would presumably, eventually go brown. Fraction 21, showed the least phytotoxicity which may have been due to its relatively high volatility as shown in Appendix 1 C or alternatively it may have been rapidly diluted within the leaf tissue due to its relatively low viscosity. The increased phytotoxicity of this fraction under conditions of high humidity may have been due to a reduction in its rate of evaporation, caused more by the closed environment rather than by an effect of humidity. One cannot, however, explain the similar effect for fraction 23 in the same way as it is virtually non-volatile (Appendix 1 C) and it may be that for this fraction, high humidity favours oil damage as suggested by Kelley (1930) though rejected later by Dallyn and Sweet (1951).

Thus fraction 21 gave almost complete disease control whilst causing negligible phytotoxicity even after 23 d.

As a final test, both the(A + H) and (N + P) components of this fraction were compared with BP85 for disease control.

e) A comparison of fraction 21 components with BP85 for the control of vine downy mildew

Discs were cut, sprayed at the 0.15 ml level and sporulation estimated in terms of the number of sporangia per disc.

Table 27 The effect of BP85 and fraction 21 components on vine downy mildew

Treatment		Sporangia/disc -3 ($\times 10$)	% Control
Control		21.5	-
Fraction 21	(A+H)	0.75	95.6
	(N+P)	16.0	25.5
BP85		19.3	10.0

Discussion

In this experiment the (A + H) component of fraction 21 again gave almost complete control of the disease as is shown in Table 27 and Plate 2 whereas the (N + P) component gave a small but significant reduction in sporulation. BP85 gave a slight reduction which was not, however, significant and again indicated the variability of the effect of oil on the disease.

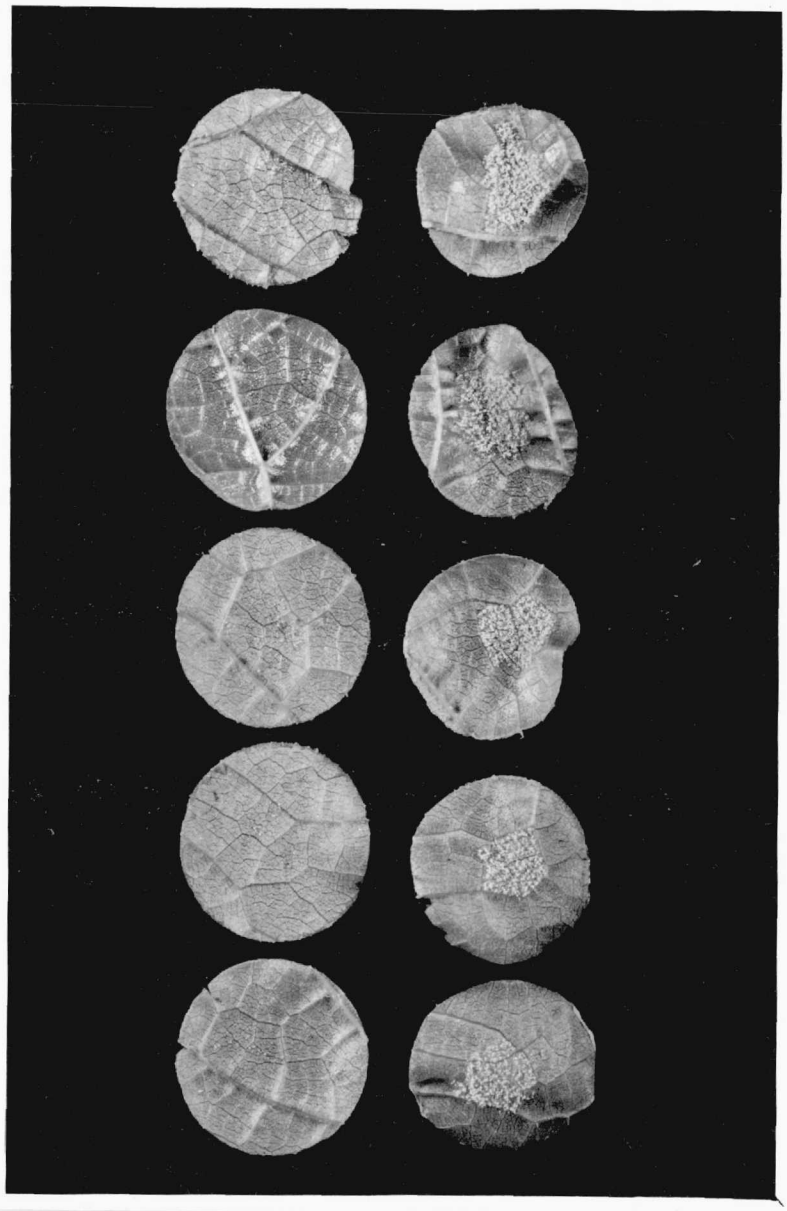


PLATE 2 Effect of fraction 21 (A + H) component on sporulation of P.viticola on vine leaf discs (L.H. discs sprayed, R.H. discs unsprayed).

2. End-point for control with (A + H) components

In previous experiments, disease control using the (A + H) Components of the less phytotoxic fractions was good but not complete. Thus, to determine the levels required to give complete control, fractions 21 and 23 were first compared for disease control.

a) A comparison of fractions 21 and 23

Discs were sprayed with the (A+H) components of fractions 21 and 23 at levels from 0.1 ml to 0.9 ml. They were inoculated and sporulation graded visually on the 0 to 5 scale as before. Results are shown in Table 28.

Table 28 The oil level of fractions 21 and 23 required for complete control of vine downy mildew

(A + H) Oil level (ml)	Sporulation grade	
	Fraction 21	Fraction 23
Control	2.4	
0.1	0.9	1.6
0.2	0.3	1.3
0.3	0	1.4
0.5	0	1.2
0.7	0	1.0
0.9	0	0.8

Discussion

Using fraction 21, complete control was obtained with the 0.3 ml level whereas with the highest level of fraction 23 used, sporulation was still quite marked. Although it is not possible to interpret grades in terms of sporangia per disc, it is clear that using fraction 23, control increased only very

gradually with quite large increases in oil level. This contradicts an earlier suggestion that this fraction might be more effective at higher levels than would be suggested from its effect at low levels (Table 25 and text). Fraction 21, however, was as effective at the lowest level of 0.1ml as fraction 23 was at the 0.9 ml level.

b) Disease control and phytotoxicity of fraction 21

Discs were sprayed with fraction 21 at levels from 0.05 to 0.60 ml, inoculated and sporulation graded as before and phytotoxicity assessed as the number of necrotic effects or oil soaked areas per disc. Results are shown in Table 29.

Table 29 Disease control and phytotoxicity of fraction 21

(A + H) OIL level (ml)	Disease grade	Damage per disc
Control	2.0	0
0.05	0.2	0
0.10	0	0.8
0.15	0	2.2
0.30	0	9.1
0.60	0	18.7

Discussion

The 0.05 ml level in this experiment gave the same order of control as the 0.2 ml level of the previous experiment suggesting that the variability of the effect of oil on leaf discs also applies to disease control. It further suggests a mechanism of action other than a simple protectant effect. Phytotoxicit was similar to previous experiments but with no damage evident at the 0.05 ml level.

It is clear, however, that even with this fraction there is little lattitude for error in oil deposits.

3. The effect of oil dilution on disease control and phytotoxicity

As shown earlier (Table 26) fraction 21 was capable of causing marked phytotoxicity in the environment of a closed Petri-dish. Whether this was due to humidity or the prevention of oil evaporation, it suggested that there might be other situations in which phytotoxicity would be more pronounced. Thus it would be desirable to dilute the fraction in a non-phytotoxic carrier oil which would reduce phytotoxicity but maintain good disease control.

Fractions 21, 22, 23 and 24 were diluted in BP85 giving concentrations of from 20% to 100% and sprayed on leaf discs at the 0.15 ml level. Viscosities of the diluted oils were measured as described previously and shown in Appendix 1 b, and used as markers for each dilution, as the value for 50% distillation temperature used in Fig. 7 would not apply here. Dilutions of fraction 21 were used against vine downy mildew whilst selected dilutions of the four fractions were compared for phytotoxicity.

Sporulation was estimated as the number of sporangia per disc as before and phytotoxicity as the total number of necrotic spots or oil soaked areas per disc. Results are shown in Tables 30 and 31.

Table 30 The effect of fraction 21 (A + H), diluted in BP85 on vine downy mildew

% (A + H)	Sporulation ($\times 10^{-3}$ Sporangia/disc)	% Control
20	18.5	20.8
40	8.5	63.5
60	3.4	85.4
80	2.9	87.5
100	2.4	89.6
Control	23.4	—

Table 31 The phytotoxicity of (A+H) components diluted in BP85

(Total number of necrotic spots and oil soaked areas/disc)

Estimation time (d)	Fraction number	% (A + H) 20	60	100
4	21	0.6	0.9	5.5
	22	0.6	2.4	4.7
	23	0.2	1.2	
	24	1.2	3.7	
13	21	1.2	2.6	6.6
	22	1.6	6.7	10.0
	23	1.9	2.7	
	24	3.1	15.4	

Discussion

The activity of fraction 21 remained at a high level down to and including a dilution of 60% after which it fell off rapidly. Even at this level, however, phytotoxicity was still evident and in terms of a whole vine leaf would probably be unacceptable. Fig. 9 shows that for disease control, activity is not directly related to the concentration of the (A + H) component, the reduction in sporulation at 60% being almost the same as 100%. The phytotoxicity of fractions 21 and 22 do, however, follow concentration more closely, although with fraction 21 phytotoxicity increases more rapidly from 60% to 100% than up to 60%. Only two dilutions of fraction 24 were tested but it is clear that the difference in phytotoxicity between the two is not related to (A + H) component concentration.

Fig. 9. The effect of (A + H) component dilution on sporulation and phytotoxicity

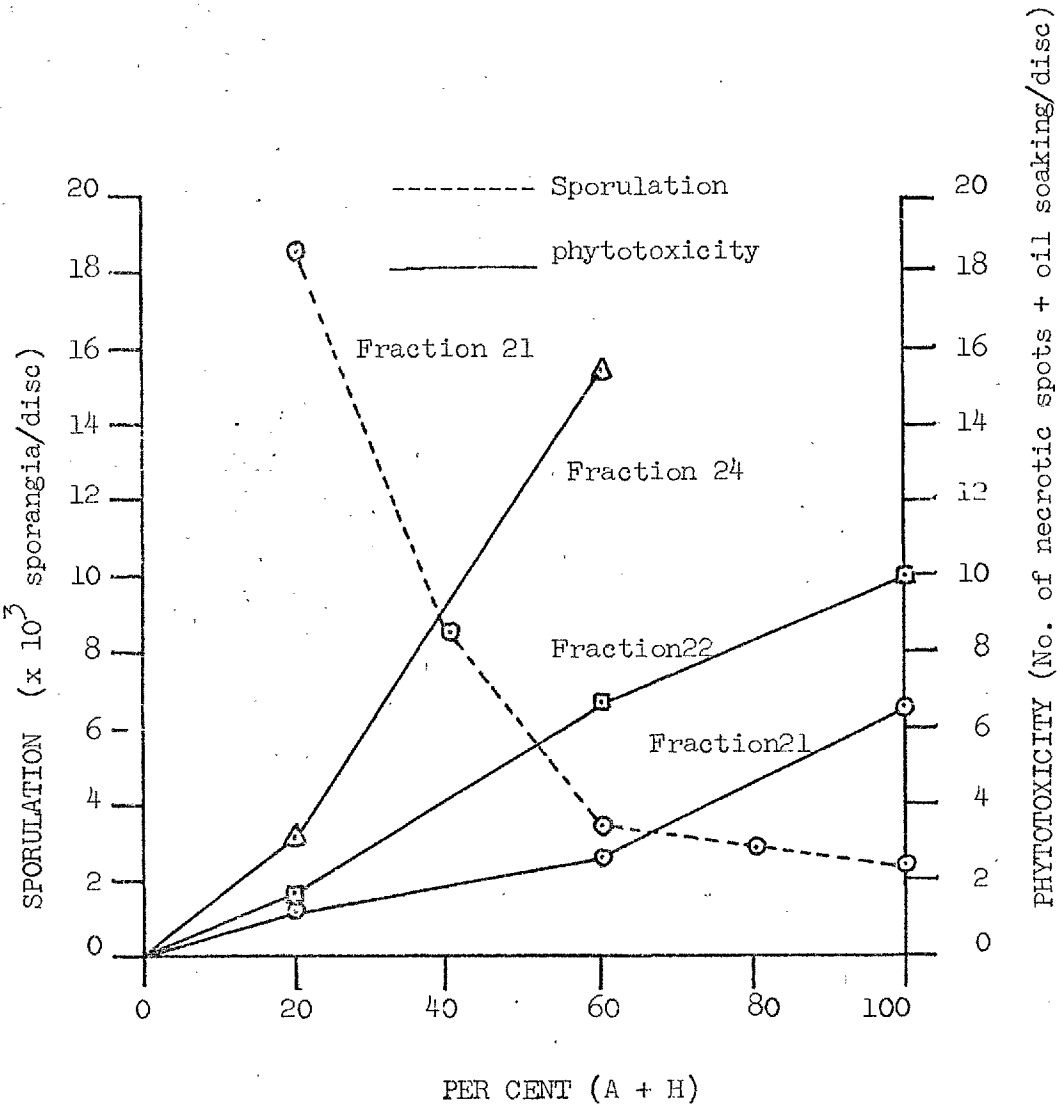


Fig. 10. A comparison of (A + H) component phytotoxicity at 60 per cent dilution

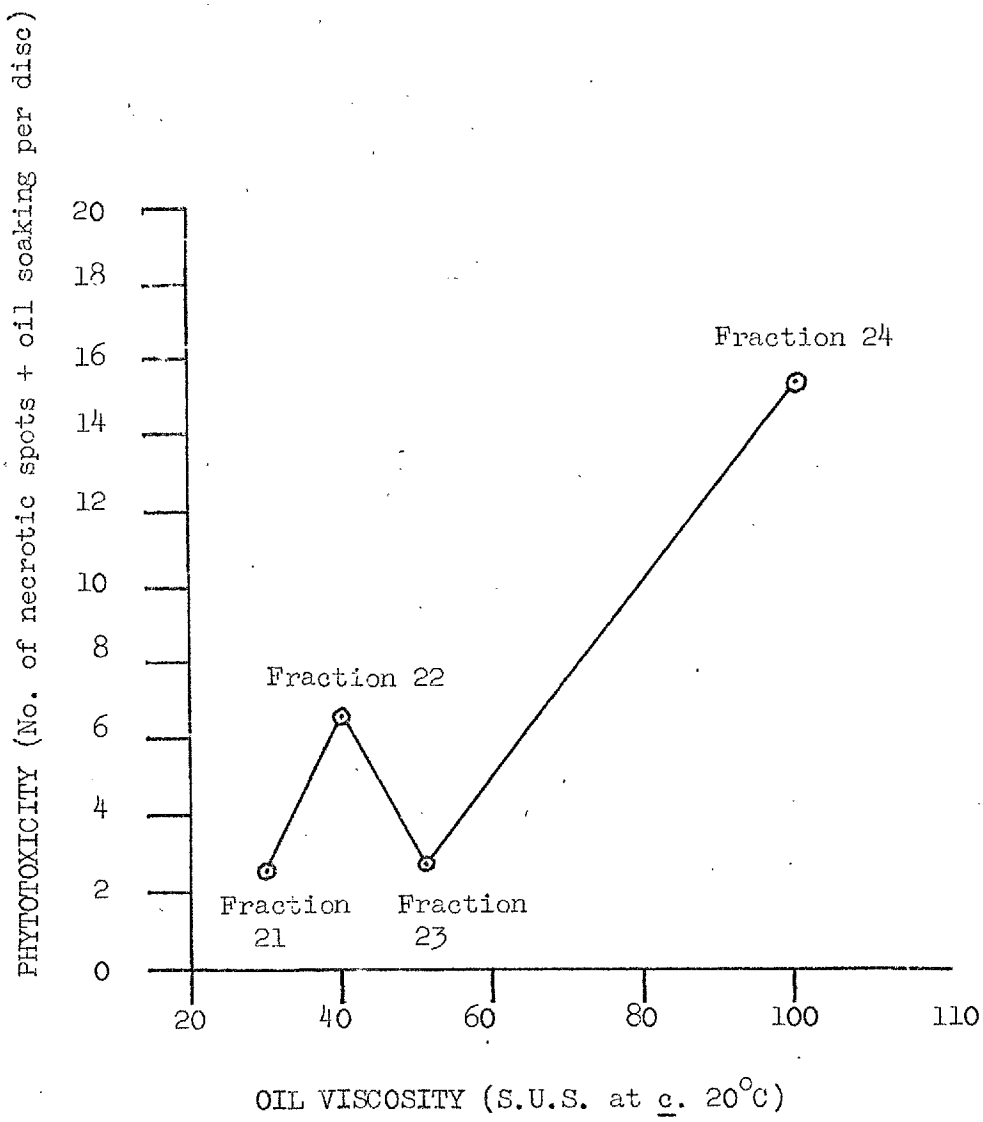


Fig. 10 shows the phytotoxicity of the four fractions at a dilution of 60% and shows clearly the variation in phytotoxicity with the different oil fractions and the two troughs of low phytotoxicity given by fractions 21 and 23.

4. The effect of a carrier-oil on the activity of (A + H) components

The previous experiment showed that anti-mildew activity was not directly related to the concentration of fraction 21, (A + H) component in BP85 carrier oil. At higher concentrations, the BP85 had little effect on activity whereas at lower concentrations, the carrier oil masked the effect of the (A + H) component. To confirm this latter effect, the crude oil fraction 21 was tested against downy mildew at levels from 0.15 ml to 0.60 ml. As the crude oil was known to contain 34.3% of the (A+H) component, if the carrier oil (i.e. the (N + P) component of fraction 21 crude oil) was having no effect, a crude oil level of 0.44 ml should give the same disease control as 0.15 ml of the (A + H) component. Thus fraction 21 crude oil was compared with fraction 21 (A + H) component together with fraction 20 and 23 crude oils at 0.15 ml. Discs were inoculated and estimated for sporulation in terms of grades and sporangia produced as before.

Table 32 The effect of crude oil fractions on vine downy mildew

Fraction number	Oil type	Oil level (ml)	Sporulation grade	Sporangia/disc $(\times 10^{-3})$	% Control
Control		—	3.1	30.4	
20	C.O.*	0.15	3.1	31.9	
21	C.O.	0.15	2.1	20.0	34.2
"	"	0.30	1.6	16.7	45.1
"	"	0.60	1.2	9.0	70.4
"	(A + H)	0.15	0.3	2.9	90.4
23	C.O.	0.15	2.4	26.0	14.5

* = crude oil

Discussion

As can be seen from Table 32 even at the 0.6 ml level the crude oil fraction 21 did not give as good control as the (A + H) component, confirming that the carrier oil was masking the activity of the latter. Neither the crude oil fractions 20 or 23 gave any marked disease control, the former presumably volatilizing before it could have any effect.

5. The eradicant action of (A + H) components

As oils are known to penetrate into leaf tissues it was possible that in doing this the (A + H) component of oils might in hibit the fungus once it had penetrated and become established. To test this the (A + H) component of fraction 21 was sprayed at the 0.15 ml level onto discs just prior to inoculation as normal and then after removing the inoculation drops at 24, 48 and 72 h . Sporulation was estimated as the number of sporangia per disc and the lesions graded in terms of the degree of browning using a scale from 1 to 3. To show that any effect was due to the (A + H) component, this was compared with (N + P) component of the same fraction.

Table 33 The effect of post-inoculation sprays of the (A + H) component of fraction 21

Treatment time (d)	Sporulation ($\times 10^{-4}$ sporangia/disc)	% Control	Lesion browning*
Control	5.70	—	1.0
0	0.95	83.3	0
1	0.70	87.7	2.7
2	0.90	84.2	1.7
3	1.20	78.9	1.5

* 1 = Light browning - oil soaking and/or brown flecks with start of browning

2 = Moderate browning - mainly brown but with some non-brown flecked tissue remaining

3 = Complete browning.

Table 34 A comparison of the (A + H) and (N + P) components of fraction 21 for eradicant activity

Time	Sporangia/disc ($\times 10^{-3}$)		% Control	
(d)	(A + H)	(N + P)	(A + H)	(N + P)
Control	14.5		—	
0	2.2	15.2	84.8	—
1	2.5	15.0	82.8	—
3	3.1	14.8	78.6	—

Discussion

When, after 3 d, the discs were transfered to high humidity conditions, the control discs showed light but consistent sporulation and the pre-inoculation treatment showed none. The post-inoculation treatments, however, showed degrees of browning in the area of the inoculation drops and were roughly graded as shown in Table 33. The 1 d treatment showed no sporulation but marked browning. The 2 d treatment showed slight sporulation at the edge of some of the inoculation areas which were otherwise browned though somewhat less than the 1 d treatment discs. The 3 d treatment showed more sporulation than the 2 d treatment with browning slightly less than 2 d. After 5 d sporulation on the controls had increased with some evident *also on the pre-inoculation treatment discs. At this time sporulation was estimated, the results being given in Table 33 As can be seen, control was maintained, in terms of a reduction in sporulation, up to 3 d after inoculation, and accompanied by much tissue browning. This was not evident on the pre-inculation treatment, there being no sign of any type of damage. Thus there would seem to have been an interaction of the oil and

the invading fungus resulting in the prevention of sporulation and browning and death of leaf tissue.

The results of the second experiment given in Table 34 showed that only the (A + H) component was capable of eradicant action, the (N + P) component in this case causing a slight increase in sporulation. This again indicates the variability of the effect of oil, previous tests with this fraction component having shown a small reduction in sporulation or no effect at all. Some browning was noticed in the (A + H) treatments but was less marked than in the first experiment suggesting that browning was not necessarily to be associated with eradicant action.

6. The persistence of the (A + H) component of fraction 21

The (A + H) component of fraction 21 was relatively volatile as shown in Appendix 1 C, this having already been suggested as an explanation for its low phytotoxicity. Volatility might also be expected to affect the persistent activity of the fraction and discs were therefore sprayed at the 0.15 ml level at 0, 1, 3, 5 and 7 d prior to inoculation. A parallel series of discs were sprayed at the same times but the five discs of each replicate washed in 20 ml of distilled water for 20 s, using a whirly mixer, to simulate the effects of heavy rainfall. The resulting leaf washings were tested against the germination of Plasmopora viticola on glass slides as described previously. Both washed and unwashed discs were inoculated and sporulations estimated visually.

Table 35 The effect of pre-inoculations sprays of fraction 21, (A + H) component on sporulation and the activity of leaf washings

Pre-treatment time	Sporulation grade		% Germination in leaf
(d)	Un-washed	Washed	washings
Control	2.10	2.10	76.4
0	0.22	0.82	77.1
1	0.54	0.68	76.9
3	0.87	0.95	76.1

Table 35 cont.

Pre-treatment time (d)	Sporulation grade		% Germination in leaf washings
	Un-washed	Washed	
5	0.93	1.01	75.9
7	0.98	1.07	76.8
Slide germination control			63.5

Discussion

Although infection was not very good the effect of washing in relation to persistence can be clearly seen. Washing the discs immediately after spraying and before inoculation caused a small but significant increase in sporulation. The increased sporulation was not due to any detectable inhibitor being washed off the discs, as germination was greater in leaf washings than in distilled water on glass slides. The longer the oil remained on the discs the less effect washing had on infection, though the activity of the oil fell off very gradually with time. These results are therefore consistent with both the volatilization of the oil and also with penetration into the leaf.

7. The effect of inoculum concentration on disease control

In two experiments discs sprayed were with the (A + H) component of fraction 21 at the 0.15 ml level and inoculated with suspensions of 40, 20, 10, 5 and 2.5 x 10^4 sporangia/ml. If the oil was acting as a simple protectant, the degree of control should be the same for all concentrations, although a positive result in this direction would not necessarily prove this hypothesis. Sporulation

was determined as the number of sporangia per disc. Results are shown in Table 36.

Table 36 The effect of inoculum concentration on disease control (sporulation given as x 10^{-3} sporangia/disc)

EXPERIMENT 1

Concentration ($\times 10^{-4}$ sporangia/ml)	40	20	10
Control	22.7	24.0	33.0
Fraction 21(A+H)	3.5	3.7	5.6
% Control	85.1	84.6	83.1

EXPERIMENT 11

Concentration ($\times 10^{-4}$ sporangia/ml)	10	5	2.5
Control	18.1	13.4	8.0
Fraction 21,(A+H)	2.7	0.6	0.4
% Control	85.1	95.5	95.4

Discussion

As shown by Skellern (1968) increasing the inoculum concentration increased sporulation up to a point after which it was reduced. Disease control decreased by 10% when the inoculum concentration was increased from 5 to 10 x 10^4 sporangia/ml. These results thus again suggest that the mechanism of action of the oil cannot be explained in terms of a simple protectant effect.

8. The effect of leaf age on disease control

Having tested both variations in the type of oil and inoculum concentration of the fungus, host variation was tested in terms of leaf age thus combining the effects of oil and fungus under variable host conditions. Discs were cut from leaves 4, 5, 6, 7 and 9 numbered sequentially from the first unfurled leaf of old seedlings, and sprayed with the (A + H) component of fraction 21 at the 0.15 ml level. Sporulation was determined as the number of sporangia per disc.

Table 37 The effect of leaf age on disease control

($\text{Sporulation given as } \times 10^{-4} \text{ sporangia/disc}$)

Leaf number	Sporulation		%
	Control	Oil	Control
4	17.3	0.42	97.6
5	16.5	0.59	96.4
6	14.0	1.05	92.5
7	9.1	1.23	86.5
9	5.7	0.98	82.5

Discussion

As Table 37 shows, leaf age had a marked effect on disease control and would perhaps account for variations in the effect of both the (N + P) and (A + H) components on disease control in previous experiments. Disease control was highest on the younger leaves and fell off with increasing leaf age. This is again difficult to explain in terms of the oil acting as a simple chemical barrier, and combined with results of previous experiments strongly suggests that the oil is acting on the host making it more resistant to the fungus. Alternatively the oil might be acting directly on the fungus inside the leaf. In this case the effect of leaf age might be due to the greater density of stomata on young leaves than on old leaves, thus making it easier for the oil

to penetrate the leaf. In an attempt to clarify this former hypothesis experiments described below were carried out to test the effect of the oil directly on the fungus and indirectly via effects on leaf diffusates.

D. The Effect of the (A + H) Components of Oil Fractions on Zoospore Behaviour on Leaf Discs

In an attempt to study zoospore behaviour the chlorine bleach technique described previously was first tested. Although bleaching was successful the disc tended to become distorted making it difficult to see clearly the stomata and any structures associated with it. The technique of Preece (1965) as described previously was much more successful and zoospores could be seen around stomata.

Discs were sprayed at the 0.15 ml level with the (A + H) component of fraction 21 and inoculated as before. After 24 h, four control and four treated discs were fixed and counts made of the number of stomata with zoospores and the number of zoospores per stoma. Three replicates of five discs were used to determine the sporulation for both oil treatment and control, counts being made of the number of sporangia per disc.

Table 38 The effect of the (A + H) component of fraction 21 on sporulation and zoospore behaviour

Treatment	Sporulation ($\times 10^{-3}$ sporangia/disc)	% Stomata with Zoospores	Average number of Zoospores/stoma
Control	15.1	76	5
Fraction 21(A+H)	0.9	74	5
% Reduction	94.0	—	—

Discussion

As can be seen from Table 38, although disease control was high there was

no significant effect of the oil treatment on either the percentage of stomata with Zoospores or the number of Zoospores per stoma. This again provides strong evidence against the chemical barrier hypothesis of oil action.

E. The Effect of the (A+H) Components of Oil Fractions on Vine Leaf Discs

Skellern (1968) reported the presence, on or in vine leaf discs, of an inhibitor of the sporangial germination of Plasmopara viticola. In an attempt to confirm this, the modified drop-diffusate technique of Bailey (1968) was used. At the same time the effect of the (A + H) components of fractions 21 and 23 on the activity of diffusates and any possible interaction with an inhibitor if present was also tested. Thus diffusates were collected from oil treated and control discs and tested, before and after boiling, on the in vitro germination of sporangia of P.viticola. As the technique involved the use of plastic Petri-dishes, the controls included drops collected from unsprayed and oil-sprayed Petri-dishes which were then tested against P.viticola on glass slides.

Diffusates were obtained by placing 0.05 ml drops of sterile distilled water in plastic Petri-dishes and allowing a 7 mm diameter leaf disc to remain with its lower surface resting on each drop for 24 h and 48 h. The leaf area exposed to a standard volume of water was about forty times greater using this method than used by Skellern (1968) so that the inhibitor, if present, should be easily demonstrated in bioassays. Diffusates were boiled for 5 min and bioassays carried out as described previously. In Experiment 1 diffusates were collected after 24 h and in Experiment 11, after 48 h. Oils were sprayed at the 0.15 ml level. Results are shown in Table 39.

Table 39 The effect of the (A + H) components of oil fractions 21 and 23 on leaf diffusate activity

Treatment	% germination of P.viticola	
	Fraction 21	Fraction 23
EXPERIMENT 1 (24 h)		
Leaf diffusate unboiled	68.8	
Leaf diffusate boiled	66.5	
(Oil + leaf) diffusates unboiled	64.3	65.1
(Oil + leaf) diffusates boiled	62.7	64.2
Control (glass slide)	51.4	
Control (Petri-dish)	49.7	
Control (oil + Petri dish)	49.9	
EXPERIMENT 11 (48 h)		
Unboiled (oil + leaf) diffusate	51.5	51.1
Boiled (oil + leaf) diffusate	50.2	50.4
Unboiled leaf diffusate	52.1	
Boiled leaf diffusate	50.7	
Glass slide control	41.2	

Discussion

None of the treatments showed any inhibition of sporangial germination whereas diffusates from both sprayed and unsprayed discs stimulated germination. The stimulatory principle(s) in the diffusates was unaffected by boiling which suggested that they might be sugars. These results confirm those of the persistence experiment where leaf washing were also found to be stimulatory to the germination of sporangia of P.viticola. The non-significance of any fungitoxic substances in vine leaf diffusates was also indicated by the work of Dunn et al.(1969) who showed that leaf washings of five plant species including vine inhibited the fungicidal activity of ethylene thiuram disulphide (E.D.T) in vitro to A. brassicicola. He further found that the principle fungicide antagonist in water soluble leaf exudates was D-glucose.

Although this experiment did not demonstrate any effect on the fungus via an action upon the host, it does not necessarily disprove the hypothesis.

F. The Effect of the (A + H) Components of Oil Fractions on Plasmopara viticola

1. The germination of sporangia of P. viticola on oil sprayed slides

Slides were sprayed with the (A + H) components of fraction 21,22 and 23 at the 0.1, 0.2 and 0.3 ml levels. The germination of sporangia was tested on these slides as described previously. Results are shown in Table 40.

Table 40 The germination of P.viticola on slides sprayed with the (A + H) components of oil fractions

Fraction number	Oil level (ml)	Percentage germination
Control	—	71.9
21	0.1	73.2
	0.2	73.8
	0.3	74.2
22	0.1	73.3
	0.2	74.1

Table 40 cont.

22	0.3	73.6
	0.1	75.1
23	0.2	73.6
	0.3	74.2

Discussion

There was no significant difference between any of the treatments and the control, emphasizing the importance of screening oils, as potential plant disease control agents, on inoculated hosts. As Calpouzos (1966) has pointed out, many phytopathogenic fungi grow well in the presence of oils which, however, control the diseases caused by the fungi.

2. The effect of oils on the sporangia of P.viticola in situ

As oils are by definition hydrophobic substances it is perhaps not surprising that the sporangia of P. viticola germinate well on oil sprayed slides. To test the direct toxic action of oils on the fungus, sporangia in situ on vine leaves were sprayed with oils and their germination then tested.

a) The effect of fraction 22 components and BP85

Discs were cut from sporulating leaves and sprayed with crude oil (C.O.) fraction 22, its (N+P) and (A+H) components at the 0.15 ml level and with BP85 at the 0.15 ml and 1.0 ml level. The treatments were left for 6 h on moist paper tissue in Petri-dishes in a 17°C lighted incubator. The sporangia were then washed off, each of the three replicates of four discs in 1.0 ml of sterile distilled water, and the germination of the sporangia tested on slides as usual. Results are shown in Table 41.

Table 41 The effect of fraction 22 components and BP85 on sporangia of P.viticola in situ

Treatment	Oil level (ml)	% germination
Control	—	89.6
Fraction 22 (C.O.)	0.15	89.0
" " (A+H)	0.15	88.7
" " (N+P)	0.15	89.4
BP85	0.15	89.9
"	1.00	89.8

Discussion

There was no significant difference between any of the treatments and the control showing that the oils had no direct toxic action on the sporangia.

b) The effect of (A + H) components

The above experiment was repeated using the (A + H) components of fraction 19, 21 and 23 at the 0.15 ml level.

Table 42 The effect of (A + H) components on sporangia of P.viticola in situ

Fraction number	% germination
Control	93.3
19	92.5
21	91.6
23	92.7

Discussion

There was again no significant differences between any of the treatments showing that the (A + H) components were not toxic to sporangia.

G. The Phytotoxicity of (A + H) Components of Oil Fractions on Whole Plants

Whole vine seedlings were sprayed with the (A + H) components of fractions 19 and 21 as described on page 48 using the Micron ULVA, giving deposits of 2.9 and 4.9 mg/100 cm^2 respectively. The plants were kept in the greenhouse at temperatures varying between c. 15°C to c. 35°C for 36 h . Phytotoxicity was mainly in the form of necrotic spots and was roughly graded on a scale from 0 to 8 i.e. no necrotic spots to coalescence of necrotic spots. Three plants were used for each treatment and the results showing the variation between leaves and plants are given in Table 43.

Table 43 Phytotoxicity of (A + H) components of fraction 19 and 21 on whole plants (grade/leaf)

Replicate	Fraction 19			Fraction 21		
	Leaf 1	Leaf 2	Leaf 3	Leaf 1	Leaf 2	Leaf 3
1	0	0	0	2	4	—
2	0	0	0	1	6	5
3	0	0	0	3	6	5

Discussion

Thus, whereas fraction 19 presumably volatilized before it could cause any phytotoxicity, fraction 21 caused very heavy phytotoxicity. The fact that damage appeared so soon at its maximum (phytotoxicity did not noticeably increase over a period of 8 d) demonstrated the vastdifference between leaf discs in the favourable conditions of a Petri-dish and whole plants in the

greenhouse at relatively high temperatures and dry conditions.

Thus the (A + H) component of fraction 21 could not possibly be used in its crude state in commercial practice.

H. Mass Spectroscopy of the (A + H) Component of Fraction 21

As the crude (A + H) components caused heavy phytotoxicity the next logical stage was to attempt a separation of fungitoxic compounds from phytotoxic compounds. As a first stage the (A + H) component of fraction 21 was analysed in a mass spectrometer to determine the main types of hydrocarbons present. The work was carried out by the Mass Spectroscopy Group, Analytical Section, British Petroleum Research Centre, Sunbury-on-Thames and the type analysis is given in Table 44.

Table 44 Type analysis of fraction 21

Hydrocarbon type	Formula	Percentage of fraction
Paraffins	Cn H2n + 2	
Mono-Cyclo-paraffins	Cn H2n	
Di-Cyclo-paraffins	Cn H2n-2	
Tri " "	Cn H2n-4	3
Tetra " "	Cn H2n-6	
Penta " "	Cn H2n-8	
Hexa " "	Cn H2n-10	
Alkyl benzenes	Cn H2n-6	11
Benzo-cyclo-paraffins	Cn H2n-8	4
Benzo-dicyclo-paraffins	Cn H2n-10	5
Naphthalene	Cn H2n-12	2
Acenaphthenes	Cn H2n-14	3
Fluorenes	Cn H2n-16	11
Phenanthrenes	Cn H2n-18	15
Pyrenes	Cn H2n-22	4
Chrysenes	Cn H2n-24	—
Benzo-thiophenes	Cn H2n-10 S	11
Dibenzo-thiophenes	Cn H2n-16 S	31
Naphthobenzo-thiophenes	Cn H2n-22 S	—

Discussion

As can be seen the analysis was very complicated with only the groups of compounds given. The sample was apparently much more complex than even the above analysis suggests, which should therefore be treated with reserve.

At this stage the work was terminated as it would require a very large screening programme, to determine which compounds were fungitoxic and which phytotoxic. The facilities necessary for this separation were not, at the time available.

SUMMARY AND DISCUSSION

Of the wide distillation range oils used, only gas oil containing a high percentage of unsaturates (c.21%) and BP85 exposed to sunlight, gave any significant control of vine downy mildew. The degree of control was only very small, however, showing that these oils could not be used on a commercial scale to control the disease. The effect of BP85 (stored in the dark) on the disease was very variable. In different experiments it was shown to reduce slightly, have no effect on or produce a slight increase in sporulation. It is possible that these variable effects on the disease might be a result of the variable effect of the oil on the plant shown by Skellern (1968). Furthermore, it was shown that the ability of the (A + H) component of oils to control the disease was affected by leaf age. The latter might thus have contributed to the variable results using BP85 against the fungus. The ability of BP85 exposed to sunlight to cause a small but significant reduction in sporulation complements the results of Skellern (1968) who found that similarly treated oil caused a significant reduction in germination of Plasmopara viticola sporangia in vitro. They also emphasize the desirability of working with fresh batches of oil of known quality as pointed out by Calpouzos (1966) when studying the action of oil on banana leaf spot.

The saturated components of the experimental oil fractions showed variable effects on the disease similar to BP85, although fraction 21 consistently gave a small reduction in sporulation. The most promising oil tested was the (A + H) component of the same fraction showing good disease control and the least phytotoxicity of all (A + H) components. There was no detectable effect on the in vitro germination of sporangia of the fungus or on sporangia in situ on the leaf and, as zoospore behaviour was not affected it is suggested that the action of the oil must be within the host leaf. It is possible that the mechanism of action of the oil is similar to that suggested by Manning (1968) for the action of oil in controlling banana leaf spot. According to this hypothesis the oil has a sufficiently large effect on the host cells as to induce the formation of a fungitoxic substance(s) which then inhibits the growth of the fungus. In the case of banana leaf spot it was suggested that the oil, which consisted mainly of unreactive, saturated hydrocarbons, was acting by physically damaging the host cells without causing their death and thus inducing the production of a fungitoxic substance. The evidence for the induction of inhibitory substances in this way is controvercial. Isolated carrot roots stored in the cold were shown by Sondheimer (1957) to accumulate a fungitoxic compound. Deverall and Vessey (1969) showed that lightly bruising the leaf of broad bean with a wooden rod produced a build-up of the phytoalexin, wyerone acid, in leaf diffusates. Cruickshank and Perrin (1963,1971), however, found no difference in the production of the phytoalexins pisatin and phaseollin between endocarp tissue of pea (Pisum sativum) and bean (Phaseolus vulgaris) respectively which has been scarified in situ and control tissue. In all these reports, rather crude methods were used to produce wounding and it may be that the oil, after it penetrates the leaf tissue, might come into such intimate contact with plant cells as to be capable of inducing the production of much higher levels of phytoalexin than by any of the above methods.

In the case of the (A + H) components, the oil consists of highly reactive, unsaturated aromatic and heterocyclic hydrocarbons. Thus instead of or in addition to the physical action on plant cells described above, there may also be a chemical effect. Salts of heavy metals such as copper and mercuric chloride are known to induce the formation of pisatin in pea pods (Cruickshank and Perrin, 1963) and it may be that the oil acts in a similar way on the leaf tissue of vine. Perrin and Cruickshank (1965) also found that sulphydryl group containing compounds were capable of inducing the production of pisatin and suggested that pisatin production might be a result of some disturbance in sulphur metabolism. The (A + H) component of fraction 21 contained about 40% of thiophenes (heterocyclic, hydrocarbon sulphides) and thus these might be responsible for causing a disturbance of the sulphur metabolism of vine leaves and thus inducing a phytoalexin. The effect of sulphidesin general on phytoalexin induction has not, however, been investigated. Nevertheless, the ability of vine leaves tc produce a phytoalexin-like substance has been demonstrated (Gadiev, 1969).

Although diffusates from oil treated leaf tissue were not inhibitory to the germination of P.viticola in vitro, it does not necessarily disprove the above hypothesis, merely suggesting that the fungitoxic compound(s), if induced, is prevented in some way from passing out of the leaf tissue, possibly by the oil itself. As the seat of action of the oil appears to be within the host leaf, the diffusion of a fungitoxic substance out into the inoculation drop would not be important. That the oil is acting within the leaf is further shown by the fact that the oil was able to reduce sporulation even when applied up to 3 d after inoculation.

Further support for the hypothesis is provided by the experiment on leaf age. Disease control was significantly greater on young leaves than on old leaves (97.6% as compared with 82.5%). Thus the oil may have produced higher levels of phytoalexin in the younger leaves than in the older ones. Little work has been carried out on the effect of leaf age on phytoalexin production though Cruickshank and Perrin (1963) showed an inverse relationship between pea pod maturity and pisatin production. Bailey (1969) also found that the amount of pisatin produced by pea leaves decreased as they underwent senescence. The inoculum concentration experiment showed that this had only a small effect on disease control, there being a small though significant difference between disease control at high and low concentrations suggesting a mechanism of action other than a simple chemical effect.

Without attempting to extract a phytoalexin from oil sprayed leaves, the above hypothesis cannot be proved. It is possible that in fact, the action of the oil within the plant is directly on the fungal mycelisim but as the fungus is an obligate parasite this would be difficult to demonstrate. The effect of leaf age on this basis could be explained by the greater penetration of oil into young developing leaves due to the larger number of stomata per unit area on these leaves than on older ones.

The activity of the (A + H) component of fraction 21 was shown to be masked at dilutions in BP85 of below 60% suggesting that due to its inherent solubility in oil and hydrophobic nature, the active principle in the component was tightly bound with the diluent. This effect was again shown by the lower activity against the disease of the crude oil fraction 21 sprayed at a level calculated to give the same amount of (A + H) component as spraying with the pure (A + H) component. The activity of this component was further shown to decrease gradually when sprayed up to 7 d before inoculation. It is possible that this was due to volatilization from the leaf surface as this was shown to be quite significant in volatility tests using filter paper. It is suggested that this volatility is partly, at least, the reason for its

relatively low phytotoxicity.

Both disease control and phytotoxicity were related to boiling range with the former increasing with decreasing boiling point until, as with fraction 19, volatility became so high that the oil did not persist long enough to have any effect. Phytotoxicity in the lower boiling range was in the form of scorching, large leaf areas becoming completely browned. As boiling point increased damage became restricted to small reddish brown necrotic spots. With further increases, oil soaked areas became more evident which gradually turned brown though with increasing boiling point the time taken for this change increased. The (A + H) component of fraction 21 came in the middle of this sequence, its boiling point not being so low as to cause rapid necrotic 'spotting' or too high as to be so persistent as to cause long term oil soaking.

The effect of environmental conditions on phytotoxicity emphasized the variable effect of oil particularly with respect to water conditions. Thus although using leaf discs, phytotoxicity was relatively low, whole plants treated with the oil and left in the greenhouse showed very heavy damage. It was clear, therefore, that in its crude state, the fraction could not be used commercially and it would involve a very lengthy screening programme to separate phytotoxic from fungitoxic compounds even if this were possible. The complexity of the fraction as shown by mass spectroscopy indicated the size of the problem.

EXPERIMENTAL SECTION II

FIELD EXPERIMENTS ON THE USE OF OILS AGAINST VINE DOWNY MILDEW

INTRODUCTION

Vine plants in the field are in a considerably different environment and may therefore differ physiologically from leaf discs in the laboratory. Thus, although BP85 does not have a consistently large effect on downy mildew of vine on leaf discs, and disease development on leaf discs and whole leaves does not obviously differ (Skellern, 1968), it was possible that oil sprayed onto attached leaves particularly under field conditions might have a sufficiently different effect from oil sprayed onleaf discs that it might control the disease. Castellani and Matta (1962) reported that a light mineral oil (Esso Process 95), when misted alone on grapes in the field at a rate of 20 kg/ha, gave a 53.9% reduction in leaf area covered by the disease. The oil did not improve the effectiveness of copper oxychloride or zineb when compared with water as a carrier but when used alone at the above rate, control was not significantly different from that using copper oxychloride (50% Cu) at 0.625 kg/ha, zineb (78% a.i.) at 1.25 kg/ha, or 1% Bordeaux mixture at 2.5 kg/ha. At the time of the first assessment (July), however, disease incidence was very low but when subsequent observations were made in September, more than 25% of the leaf area had been infected and the oil based treatments were much more lightly infected than the water based treatments.

In view of this report, field experiments were carried out with gas oil, BP85 and 150 neutral in the Gironde region of France during the summer of 1969. A section of vinyard lying outside Saint Foie La Grande and close to the river Dordogne, was made available by M.A. Morize

A plot experiment was used to test the effect of the oils on natural infection and disease development of vine downy mildew. Studies were also made on the effect of the oils on established lesions and on artifical inoculations.

RESULTS

A. Plot Experiment

This was set up to allow for variations in natural infection, the treatment of selected leaves, though less time consuming, being thought to be inadequate, particularly if infection was low. The treatments were gas oil, BP85 and 150 neutral applied at rates of 10, 30 and 60 l/ha at 2wk intervals using the Minimicron. The same oils were also applied at 15 l/ha at 1 wk intervals, thus applying the same amount of oil in 2 wk as the 30 l/ha/2 wk treatments, and testing the effect of application time. The use of the Minimicron is illustrated in Plates 3a and 3b. Oil treatments were compared with the conventional copper oxychloride (Fongipron) suspended in water and applied at the rate of 2501/ha (5kg/ha) using the mechanical knapsack sprayer (Technoma) illustrated in Plate 4.

In initial applications the Minimicron was held just above knee height and directed obliquely with an up and down motion while walking down the rows, to ensure complete coverage of the plants. It was found later that maximum deposition on the undersurface of levels was produced when the Minimicron was held as close as possible to the ground with the spray directed upwards into the leaf canopy (Appendix Id).

The section of vinyard made available for the experiment consisted of six rows each of between ninety six and ninety eight plants which were a mixture of the cultivars Merlot, Cabernet and Cote Rouge. Only the latter, predominant cultivar was used in the experiment and marked to distinguish it from the others. Due to the large number of treatments, the normal plot arrangement of three rows with the central one used for estimations, could not be used. Each row was divided into eight sections or plots giving a total of forty eight, allowing sixteen treatments with three replicates per treatment.

PLATE 3a

Minimicron - front view



PLATE 3b

Minimicron - side view



PLATE 4

Technoma Knapsack sprayer



Due to the uneven distribution of cv. Cote Rouge, the plots were of unequal size but were designed so that each consisted of at least six plants of this cultivar, the four most central ones in each plot being used for disease assessment and there being at least two other plants at either end. These protected the test plants from spray from adjacent plots. Treatments, including nine untreated control replicates, were randomized throughout the plots.

Before the experimental area could be marked out and treated, heavy rainfall caused a severe attack of downy mildew with between 40% and 60% leaves infected as illustrated in Plate 5a. As the flowers were also badly attacked the results of the treatments were assessed in terms of the number of leaves remaining on the plants in mid-August as shown in Table 45.

Table 45 Average number of leaves remaining on plants in mid-August after treatment with mineral oils

Treatment			Average number of leaves per plant
Oil	Rate (l/ha)	Wk between sprays	
Gas oil	10	2	55
" "	30	"	67
" "	60	"	78
BP85	10	"	69
"	30	"	78
"	60	"	85
150 Neutral	10	"	96
"	30	"	76
"	60	"	81

PLATE 5a

Heavy attack of vine downy mildew early in July.



PLATE 5b

Almost complete defoliation by early September.

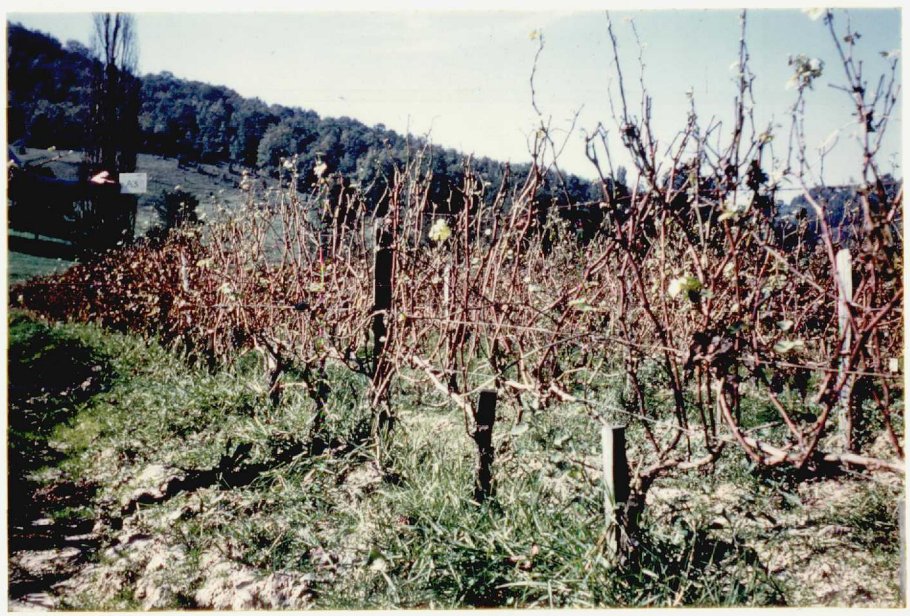


Table 45 cont.

Treatment			Average number of leaves per plant
Oil	Rate (l/ha)	Wk between sprays	
Gas oil	15	1	65
BP85	"	"	80
150 Neutral	"	"	77
Copper Oxychloride	250	2	217
Control			72

Discussion

There was no significant difference between the oil treatments and untreated controls whereas there were between two and three times as many leaves on the copper oxychloride treated plants. By early September defoliation was almost complete as shown in Plate 5b. Thus the experiment showed clearly that none of the oils were able to check the progress of the infection.

B. Effect of Oils on Lesions at 'Oil-Spot' Stage

The following experiments were carried out using selected leaves with lesions at various stages of development.

Experiment 1

To determine more clearly whether the oils had any eradicant action on the disease, established lesions at the 'oil-spot' stage before sporulation, illustrated in Plate 6a, were sprayed and observed for sporulation shown in Plate 6b. Leaves with at least three lesions were tagged and each of three lesions marked on each leaf with three small dots using a felt-tip pen. This method of marking had been shown previously to have no effect on lesion development

PLATE 6a

'Oil-spot' stage of infection by vine downy mildew

PLATE 6b

Sporulation on undersurface of leaf

The oils used were normal gas oil containing about 21% (A + H) component, gas oil with the (A + H) component removed, BP85 and 150 neutral and were applied using the Micron ULVA. The leaves were held so that the light cloud of oil droplets produced by the Micron ULVA drifted over the undersurface of each leaf producing a barely visible deposit. Fifteen leaves were used for each treatment with fifteen control leaves interspersed with each treatment.

Sporulation recorded as either 1 (= none or slight) or 2 (= moderate to heavy) at 2 d and 4 d after treatment is given in Table 46.

Table 46 The effect of oils on sporulation of 'oil-spot' lesions

(no. of lesions/grade)

	Day 2		Day 4	
Treatment	Grade 1	Grade 2	Grade 1	Grade 2
Gas oil	5	45	0	45
Control	4	41	0	45
Gas oil - (A+H)	3	42	0	45
Control	4	41	0	45
BP85	6	39	0	45
Control	4	41	0	45
150 Neutral	7	38	0	45
Control	3	42	0	45

Discussion

None of the oils had any significant effect on sporulation at 2 d and by 4 d all lesions were sporulating moderately to heavily. Lesions eventually turned brown as shown in Plate 7.

PLATE 7

Vine downy mildew lesion (ringed) at brown 'dead' stage.



Experiment 2

The above experiment was repeated except that gas oil without the (A + H) component was excluded and a very heavy deposit of oil was allowed to accumulate on the leaves. With gas oil this immediately produced small oil soaked patches irregularly scattered over the leaf. Observations of lesions at 4 d showed that as in previous experiment, all had sporulated heavily.

Experiment 3

During periods of dry weather, oil-spot lesions did not sporulate immediately. This allowed the oils to be sprayed onto the lesions and to be left for prolonged periods of time before testing for sporulation. Thus the three oils, 150 neutral, BP85 and gas oil were applied lightly and left for 2, 4, 6 and 8 d. At this time the leaves were enclosed in polythene bags to induce sporulation.

After 2 d all lesions had sporulated moderately to heavily. This showed that even when allowed to remain on leaves for sufficient time to penetrate the leaf, the oils did not prevent sporulation.

C. The Effect of Oils on Artificial Infections of Vine Downy Mildew

To test whether the oils acted as protectants, leaves were sprayed and then artifically inoculated. The leaves used in the experiment were situated on new shoots which had been kept in polythene bags for 1 wk to prevent natural infection of the leaves and to induce sporulation of any latent infections. Gas oil, BP85 and 150 Neutral were applied lightly as before except that only half leaves were sprayed. Sixteen vines of cv. Cote Rouge were selected and divided into four sections. Four young leaves were tagged on each plant and one plant in each section used for each of three oils with the fourth used as a control. When applying the oils, the

test leaves in the immediate vicinity were enclosed in polythene bags to prevent the oil spray from drifting on to them.

Sporangial inoculum was obtained by enclosing leaves with non-sporulating lesions in polythene bags and leaving them overnight, leaves with sporulating lesions being collected early the next day. The sporangia were washed off and the suspension diluted to a concentration of 5×10^5 sporangia/ml. The test leaves were then sprayed with the oils after which they were inoculated with the sporangial suspension using a 'Jet-Pak' sprayer. They were enclosed in polythene bags and left for 24 h after which the bags were removed. After a further 6 d the leaves were enclosed in polythene bags again and left overnight. Sporulation was assessed as the percentage leaf area infected using a scale from 0 to 5 as shown below.

0 = no sporulation
1 = 1 - 10% leaf area sporulating
2 = 11 - 50% " " "
3 = 51 - 90% " " "
4 = 91 - 99% " " "
5 = 100% " " "

The grades were calibrated against percentage leaf area in the same way as with the Horsfall-Barratt (1945) grading system. Average grades were obtained in each plot for each oil treatment-control combination and converted to percentages. The results are given in Table 47.

Table 47 The effect of oils on the infection of vine leaves by *Plasmopora viticola*

Treatment	% Leaf area infected	% Control
Gas oil	74	0
Control	74	
BP85	27.3	43.5
Control	62.7	

Table 47 cont.

Treatment	% Leaf area infected	% Control
150 Neutral	62.0	0
Control	62.0	

Discussion

In contrast to the 'oil-spot' lesion symptons shown in Plate 6a, the fungus sporulated well over the whole surface of the control half leaves. These symptoms were similar to those produced by infections later in the season. This suggests that the oil spot lesion is caused by isolated infections due to the low inoculum early in the season.

Of the three oils tested, BP85 was the only one to produce a reduction in sporulation, giving an average of 43.5% control. The effect was very variable, replicates ranging from 0% to c. 80% and was thus similar, though to a much smaller degree to that produced by BP85 on leaf discs.

The degree of control is similar to that obtained by Castellani and Matta (1962), this being a 53.9% reduction in leaf area covered by the disease over the whole plant. In the present experiment, control was similar to that obtained on leaf discs (37.3%), although the variation from 0 to c. 80% suggests that the difference in effect of BP85 on leaves in the field as compared with leaf discs in the laboratory might be sufficient to increase the effectiveness of the oil against the disease. The variation between replicates may have been the result of the variations in the effect of oil on whole leaves reported by Skellern (1968) and more specifically, the effect of leaf age on disease control found for the (A + H) component of oil fraction 21 described in Section I.

SUMMARY AND CONCLUSIONS

BP85 caused a significant reduction in infection of vines by P.viticola of 43.5%. Neither gas oil or 150 Neutral had any effect. However, BP85 did not prevent the sporulation of established lesions nor was it able to check vinyard infection and prevent defoliation once the vines were initially infected. The inability of gas oil to control the disease is likely to have been due to the high volatility of this oil (Appendix Ic). The lack of effect with 150 Neutral was probably due to the high viscosity and/or distillation temperature of this oil, as a similar inverse relationship between distillation temperature and disease control was found with the (A + H) components of oil fractions (Section I).

Although Castellani and Matta (1962) reported that oil alone was as effective as copper oxychloride, the application rate of the latter used by these workers of 0.625 Kg/ha was much lower than the normal rate of c. 5 Kg/ha (personal communication, British Petroleum Co.). In these circumstances therefore, it is not surprising that given the ability of oil to cause a 53.9% reduction in diseas incidence and the reduced application rate for copper oxychloride, there should be little difference between the latter and oil.

The variability of the control of the disease with BP85 and the low average values obtained show that the oil could not be used alone to control the disease. In contrast to Sigatoka leaf spot of bananas where oil is an effective eradicant, with vine downy mildew the oil is of no use in this respect.

EXPERIMENTAL SECTION III

THE USE OF OILS TO CONTROL CUCUMBER POWDERY MILDEW

INTRODUCTION

The ability of mineral oils, in oil-water emulsions, to affect adversely the development of powdery mildew diseases has been reported previously (Barker and Lees, 1914 McWorter, 1927; Guba, 1928/Martin and Salmon 1931). Investigations into the use of oil alone, however, were only commenced following the commercial development of oils for use against Sigatoka leaf spot of bananas. Thus, during field trials on the use of these oils as carriers for conventional fungicides against vine downy mildew, Castellani and Matta (1962) noticed that plants treated with oil formulation were almost completely free from powdery mildew infection, suggesting that oil alone might be effective against these diseases. Further experiments (Castellani and Matta, 1964) confirmed this and showed that powdery mildew diseases of cucumber and wheat, as well as vine, could be effectively controlled with a light mineral oil (Esso Process 95) at rates of between 10 and 20 mg/100 cm^2, without causing any obvious signs of phytotoxicity. Calponzos (1960) similarly showed that mineral oil alone effectively controlled powdery mildew on poinsettia plants. When applied as a single spray equivalent to 5 gal/ac, to plants inoculated 3 d earlier with an Oidium species, no symptoms developed on sprayed leaf surfaces of treated plants until 17 d after the spray application whereas the unsprayed plants were heavily diseased on the fifth day. Synthetic oils (polybutenes) have also been shown (Fisher, 1959; Fisher et al., 1960) to control powdery mildew on roses and cucumber when sprayed to run-off as 1% emulsions in water. The resulting residues left after the water had evaporated were sufficient to prevent new infections without causing phytotoxicity, and were also visually preferable to Karathane (Dinocap) deposits.

Thus the use of mineral oils against powdery mildew diseases seemed worthy of further investigation, particularly with regard to the physical and/or chemical characteristics of oils associated with anti-mildew activity.

RESULTS

A. The Use of Leaf Discs for Tests against Mildew

The use of leaf discs allowed all treatments to be replicated on each leaf used, thus reducing experimental variation. They were also much more convenient to spray and inoculate than were whole plants, these being too large to be conveniently used with the Potter Tower. Tests were therefore carried out to determine how well they survived under different conditions.

1. The survival of leaf discs on water

10mm leaf discs were cut from both young expanding leaves and mature leaves which had completed expanding and were floated, lower surface down, on distilled water in Petri-dishes. The effect of three environments were tested:-

I An illuminated cabinet at 17°C

II An illuminated cabinet constructed on the laboratory bench with temperatures varying between c. 20°C and c. 35°C

III A greenhouse with supplementary lighting and temperatures varying between c. 15°C and c. 35°C

6 d after cutting the discs, those in environment I cut from young leaves were still green and healthy whereas those cut from mature leaves were noticeably yellow. In environment II even the discs cut from young discs were slightly yellow whilst those cut from mature leaves were completely yellow. Discs maintained in environment III showed little change.

At 8 d only those discs cut from young leaves and maintained in environment I and III were still green without any signs of yellowing.

Discussion

It was clear from these observations that leaf discs would survive for up to 8 d, which was the normal time required for mildew development from

inoculation to sporulation, only when cut from young expanding leaves and maintained either in the relatively cool conditions of a 17°C illuminated cabinet, or under conditions of high light as in a greenhouse.

2. The effect of growth stimulants on leaf disc survival and mildew development

Kinetin and benzimidazole, both of which have been shown to delay the senescence of detached leaf pieces (Person et al., 1957; Richmond and Lang, 1957), were made up in distilled water and compared at 1, 10, 100&500 parts/10^6 with tapwater and distilled water for their effect on the survival of leaf discs. As Kinetin has also been shown by Dekker (1963) to check completely the development of cucumber powdery mildew when inoculated leaf discs were floated on solutions of 10 to 20 parts/10^6, the effects of the above treatments were also tested on the disease. For each treatment five inoculated discs and five control discs were floated on 15 ml of each liquid in Petri-dishes. The state of the discs were observed at 13 d and 18 d.

At 13 d, infected discs on distilled water were completely decoloured under complete coverings of sporulating mycelium whereas infected discs on tap-water were noticeably green under the mycelium, indicating that tap-water provided some form of nutrient and delayed senescence. This was confirmed on uninfected discs which were noticeably greener than those on distilled water. Infected discs on 1 part/10^6 Kinetin supported heavy sporulation and were still noticeably green, as with tap-water, but were disease free at the extreme edges of the discs. Discs on 1 part/10^6 benzimidazole, although supporting good sporulation were completely senescent (white) beneath the mycelium. On Kinetin at 10 parts/10^6 sporulation was noticeably reduced whereas benzimidazole at 10 parts/10^6 had no noticeable effect on the disease. The discs were greener than at 1 part/10^6 but less so than Kinetin at 1 part/10^6. Control discs on benzimidazole at 10 parts/10^6 were the same as those on distilled water but not

as green as those on tap-water. At 100 parts/10^6, Kinetin completely prevented infection with the discs yellow or colourless. Benzimidazole, also at 100 parts/10^6 markedly reduced infection allowing very little sporulation though the discs themselves were much greener than those on Kinetin at the same concentration.

At 18 d, of the control discs, those on tap-water were still the greenest with very little difference between distilled water and Kinetin or benzimidazole at 1 part/10^6. Of the infected discs, only those on 1 part/10^6 Kinetin showed any green.

Discussion

Although Kinetin at 1 part/10^6 delayed the senescence of infected discs rather longer than tap-water, the difference was so little with the Kinetin evan at this concentration affecting the disease at the edges of the discs, that tap-water was used in all further experiments.

3. The maintainance of leaf discs on water-agar

Discs floating on water were inconvenient to handle particularly when transfering them from laboratory to greenhouse for inoculation. Water-agar was thought likely to provide much more stable conditions enabling rapid handling of the plates. To determine the concentration most suitable, Oxoid No.1 ion agar was made up with distilled water and compared at 0.25, 0.5, 1.0 and 2.0% in Petri-dishes. Four leaf discs were placed in each plate and the experiment carried out on the laboratory bench at room temperature.

After 24 h the agar at all concentrations had dried slightly below its original level. On the 2% agar two discs were slightly shrivelled with the others turgid but dry at their edges. On the 1% agar all discs were turgid though again slightly dried at their edges whereas on both 0.5 and 0.25% agar, all discs were completely turgid and remained so for up to 8 d provided the

dishes were kept closed.

Discussion

Dilute water-agar at 0 25% was thus suitably stable to enable easy handling and sufficiently moist to prevent discs from drying.

B. The Effect of BP85 on Mildew using Leaf Discs

Leaf discs were used for the majority of experiments, with whole plants used as a final test of the oil against the disease.

1. A comparison of BP85 oil components and the development of a technique to reduce conidial deposit variation

In preliminary tests it was shown that BP85 reduced the infection of leaf discs by cucumber powdery mildew when sprayed immediately before inoculation at rates of between 0.4 ml and 1.2 ml, using the side-arm technique with the Potter Tower as described previously. As a preliminary test of the effect of different oil types, the same oil was compared with and without its unsaturated hydrocarbon component (BP85 whole oil = W.O and BP85 (N+P) respectively) and also with a 50% cut of BP85 into high and low boiling point components ("residue" and "overheads" respectively). Each oil was sprayed at the 0.4, 0.8 and 1.2 ml levels and each treatment consisting of ten replicate discs maintained on water-agar was contained in separate Petri-dishes. For inoculation purposes the dishes were arranged in a circular settling tower with five control dishes of ten discs each arranged equidistantly among the treatments. The discs were inoculated and disease assessment carried out at 8 d as described previously. Results are shown in Table 47.

Table 47 The effect of BP85 oil components on cucumber powdery mildew - first arrangement

Treatment	Oil level (ml)	% Disease Cover	% Control
Control 1	—	100	—
" 2	—	100	—
" 3	—	100	—
" 4	—	100	—
" 5	—	100	—
BP85 (W.O)	0.4	88.0	12.0
	0.8	18.0	82.0
	1.2	1.9	98.1
BP85 (N + P)	0.4	91.6	8.4
	0.8	75.0	25.0
	1.2	26.0	74.0
BP85 "Overheads" (O)	0.4	91.6	9.0
	0.8	65.0	35.0
	1.2	4.7	95.3
BP85 "residue" (R)	0.4	93.6	6.4
	0.8	9.0	91.0
	1.2	1.5	98.5

The variation in the density of conidia deposited in the tower in this experiment was quite high as determined by visual assessment of microscope slides

placed at various positions among the treatments. As this might not have been accounted for by the five control dishes and thus give rise to spurious results due to an effect of inoculum level, the experiment was repeated with controls arranged as follows. Instead of maintaining the controls in separate dishes, each treatment dish containing ten unsprayed control discs as well as the ten treated discs.

Discs were cut and the ten treatment discs arranged over half the area of water-agar in each Petri-dish whilst the ten control discs for each treatment were kept separate. After spraying, the control dishes were replaced in the treatment dishes on the other half of the agar in each dish. The discs were then inoculated and disease assessed as before. Results are given in Table 48.

Table 48 The effect of BP85 oil components on cucumber powdery mildew - second arrangement

Treatment	Oil level (ml)	% Disease cover	% Control
	0.4	83.0	17.0
	Control	100	—
BP85 (W.O.)	0.8	7.6	92.4
	Control	100	—
	1.2	3.1	96.9
	Control	100	—
	0.4	79.5	20.5
	Control	100	—
BP85 (N + P)	0.8	26.0	74.0
	Control	100	—

Table 48 cont.

Treatment	Oil level (ml)	% Disease cover	% Control
BP85 (N + P)	1.2	8.2	91.8
	Control	100	—
	0.4	83.0	17.0
	Control	100	—
BP85 "overheads" (O)	0.8	35.0	65.0
	Control	100	—
	1.2	6.6	93.6
	Control	100	—
	0.4	59.0	41.0
	Control	100	—
BP85 "residue" (R)	0.8	16.0	84.0
	Control	100	—
	1.2	2.8	97.2
	Control	100	—

On comparing the results obtained with the four oils it can be seen that there are discrepancies in the values for disease control both between the different oil levels and between the two experimental arrangements. The relative abilities of the oils to control the disease in both experiments are

compared in Table 49.

Table 49 The relative abilities of the oils to control cucumber powdery mildew in the first two experimental arrangements

Oil level (ml)	Experimental design	
	Controls in separate dishes	Controls in all dishes
0.4	W.O. > N + P; O > R	R > N + P > O; W.O.
0.8	R > W.O. > O > N + P	W.O. > R > N + P O
1.2	R; W.O. > O > N + P	R; W.O. > O > N + P

The validity of the second arrangement depended on the assumption that variations in the density of conidia would be reflected in the values given for the disease on control discs. As disease assessments were only based on the percentage leaf disc area covered with mycelium, any effects of increased inoculum deposit on sporulation after complete coverage had been reached would not be detected. Thus it was possible that although all control discs might show almost identical coverage, the actual deposit rates in each case might vary considerably. To test whether this was significant, the experiment was repeated with the discs arranged so that all treatment replicates received the same variation in conidial deposit. This was accomplished by arranging the discs after treatment into ten replicate units each composed of one disc from each of the treatments used in the experiment and one from the control. Thus all the treatments and control replicates were scattered uniformly over the inoculation area rather than being confined to restricted areas and the inoculation proceedure was thus equivalent to the hypothetical inoculation of each disc with the same density of conidia. Further details of the proceedure are given in Materials and Methods part B.3 a) 11) and the results of the experiment in Table 50 with the relative abilities

of the oils to control the disease in Table 51.

Table 50 The effect of BP85 oil components on cucumber powdery mildew - third arrangement

Treatment	Oil level (ml)	% Disease cover	% Control
Control	—	100	—
BP85 (W.O.)	0.4	94.8	5.2
	0.8	89.8	10.2
	1.2	85.6	14.4
BP85 (N + P)	0.4	94.8	5.2
	0.8	90.4	9.6
	1.2	86.4	13.6
BP85 "overheads" (O)	0.4	89.0	11.0
	0.8	86.4	13.6
	1.2	83.0	17.0
BP85 "residue" (R)	0.4	96.1	3.0
	0.8	94.8	5.2
	1.2	91.0	9.0

Table 51 The relative abilities of BP85 oil components to control cucumber powdery mildew in the third arrangement

Oil level (ml)	Relative abilities of oils to control disease
0.4	O > N + P; W.O. > R
0.8	O > N + P; W.O. > R

Discussion

The final arrangement for inoculation again gave different results for the relative abilities of the four oils to control the disease from those obtained with the first two arrangements although results were consistent at the three oil levels. Assuming this last method to have completely overcome the effects of variation in the density of conidia, the second arrangement involving controls in the same dishes as the treatments was clearly not sufficient to account for this variation. The final method, although being tedious, was therefore used in all further experiments.

The difference between the degree of control obtained in the first two and the last experiment was considerable. Whereas the control obtained with normal BP85 at the 1.2 ml level in the first two experiments was 98.1% and 96.9%, the same treatment in the last experiment gave only 14.4% control. Assuming this to be the result of a variation in the density of conidia used, the control obtained by both Calponzos (1960) and Castellani and Matta (1964) could have been due to the use of very low inoculum levels. The latter workers obtained a reduction in mildew "spots" on cucumber leaves of 95.4% with an oil level of 8 mg/100 cm^2 whereas in the first two experiments described above, equivalent control was only obtained with an oil level of 63 mg/100 cm^2. The effect of inoculum level on the control of cucumber powdery mildew with oil was therefore investigated in a later experiment.

Although control was poor and differences between treatments small in the third experiment, a pattern in the effect of oil type was evident. Thus the "residue" component which had the highest boiling point and viscosity was the least effective oil whereas the "overheads" component with the lowest boiling point and viscosity was the most effective oil. Normal BP85 (whole oil) and BP85 (N + P) with boiling points and viscosities between the "residue" and "overheads" components were also intermediary in their effect on the disease.

There was no significant difference between BP85 (whole oil) and BP85 (N + P) indicating that the small amount of unsaturated hydrocarbons in the normal BP85 (4 - 6%) did not account for the degree of control obtained.

2. A comparison of BP85 with other wide distillation range oils

The last experiment suggested that oils of low boiling point and viscosity would be more effective in controlling the disease than those of high boiling point and high viscosity. BP85 was therefore compared with two oils of widely differing specifications. The 50% distillation temperatures or boiling points of the oils viz. gas oil, BP85 and 150 neutral as given in Table 3, were 265°C, 392°C and 428°C respectively with viscosities of 2.86, 16.13 and 32.22 centistokes at 37.8°C. These were sprayed at the 0.4, 0.8 and 1.2 ml levels and discs inoculated and disease assessed as before. Results are given in Table 52 and Fig.11.

Table 52 A comparison of BP85 with gas oil and 150 neutral for control of cucumber powdery mildew

Treatment	Oil level (ml)	% Disease cover	% Control
Control	—	100	—
Gas oil	0.4	88.0	12.0
	0.8	59.0	41.0
	1.2	1.5	98.5
BP85	0.4	94.8	5.2
	0.8	89.8	10.2
	1.2	85.6	14.4
150 neutral	0.4	97.3	2.7
	0.8	94.8	5.2
	1.2	94.4	5.6

Discussion

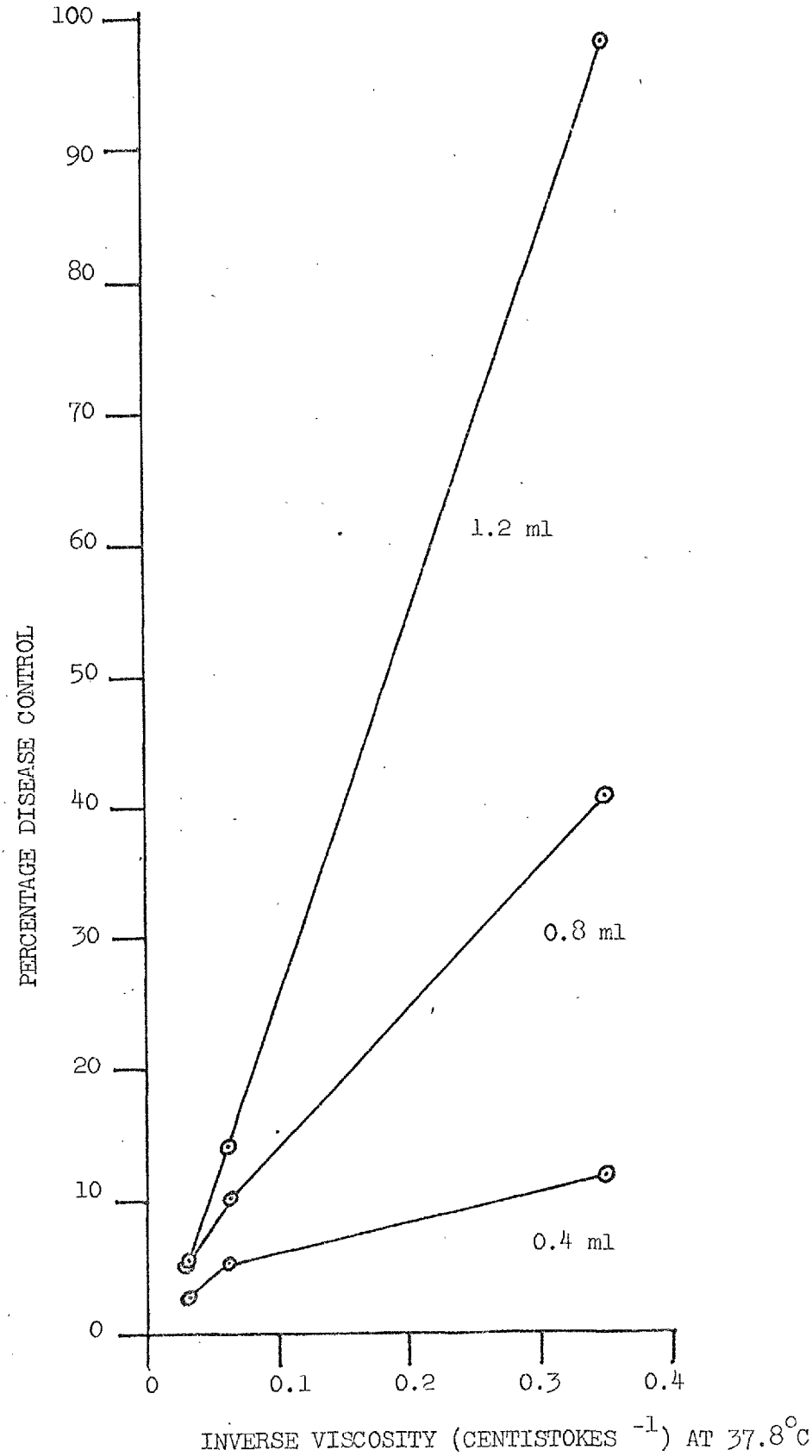
Gas oil was markedly superior to BP85 and 150 neutral, particularly at the higher levels of 0.8 and 1.2 ml. As gas oil was shown to be highly volatile (Appendix Ic) the relatively poor control at the lower levels may have been due to the lack of persistence of the oil, sufficient still remaining at the high levels to give good control.

The inverse relationship between disease control and viscosity as determined at 37.8°C is shown in Fig.11. At the 1.2 ml level the relationship is linear within the viscosity range encompassed by the three oils though too much emphasis should not be placed on this conclusion due to the lack of points between BP85 and gas oil. The error of points on the 1.2 ml line was, however, small since they all occurred at the more accurate extremes of the disease scale. The curvilinear relationship at the 0.4 and 0.8 ml levels may have been due to the high volatility of gas oil as suggested above. Thus at the 1.2 ml level volatilization would cause a smaller percentage reduction in leaf area protected than at the 0.8 and 0.4 ml levels due to the higher ratio of oil deposit volume to leaf area covered at the high level.

The linearity of the inverse relationship between disease control and viscosity depends on the temperature at which the latter is determined. This is clearly illustrated by a comparison of the ratios of the inverse viscosity changes between 150 neutral and BP85 and between BP85 and gas oil. From viscosity values determined at 21.1°C, 37.8°C and 100°C, these are respectively 1 to 12.3, 1 to 9.3 and 1 to 5.9, the ratio for disease control being 1 to 9. That disease control was slightly more closely related to viscosity measurements at 37.8°C rather than 21.1°C, even though this latter temperature is presumably very close to the normal experimental working temperature of the oils in the above experiment, suggests that viscosity is not a complete measure of the toxicity of oil to the disease.

The relationship between distillation range as represented by the 50% boiling

Fig. 11 The relationship between the inverse of viscosity and disease control



point of each oil and disease control is even more curvilinear than for inverse viscosity at 100°C. The ratio in this case is 1 to 3.5 caused by the increasingly curvilinear relationship between viscosity and 50% boiling point (Appendix Ib).

3. The effect of BP85 on disease coverage and chlorophyll content of leaf discs infected with cucumber powdery mildew

As earlier experiments had shown that leaf discs survived only just long enough to complete an infection cycle, it was thought that this would make senescence as measured by the chlorophyll content of leaf discs, a particularly sensitive test of oil phytotoxicity.

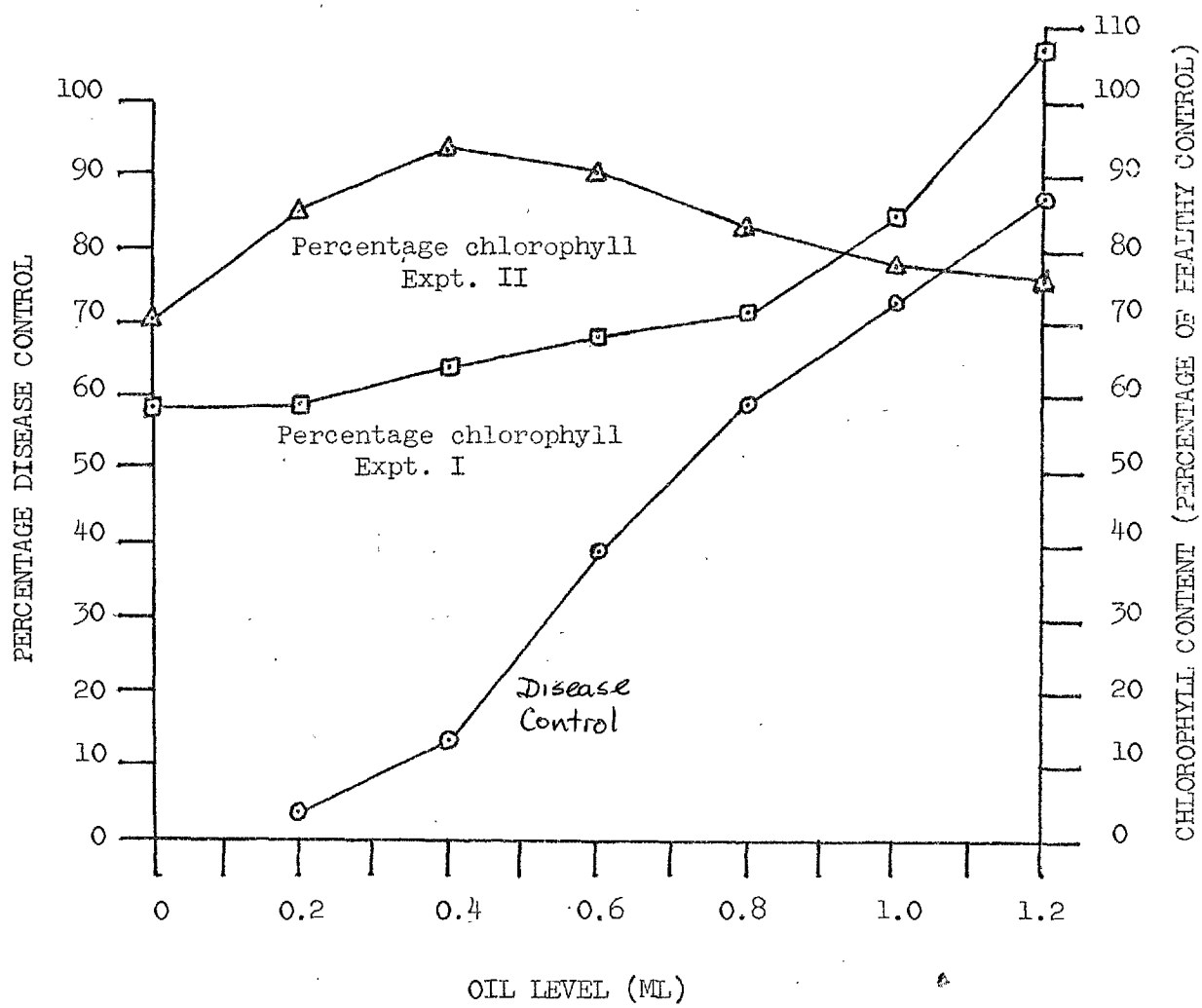
Experiment I

Discs were cut, sprayed with BP85 at levels of from 0.2 ml to 1.2 ml as before. Each treatment consisted of twenty replicate discs which were inoculated as usual. Discs were assessed for mildew at 8 d and the chlorophyll content of each disc measured using the chlorophyll assay method described previously. Controls consisted of both healthy untreated and unsprayed infected discs. Results are given in Table 53 and Fig. 12

Table 53 Disease coverage and chlorophyll content of leaf discs sprayed with BP85 and inoculated with S. fuliginea

Treatment	Oil level (ml)	% Disease cover	Chlorophyll (D. at 665mμ)
Healthy Control	—	—	0.114
Unsprayed Control	—	99	0.067
BP85	0 2	96.2	0.067
	0.4	86.3	0.073
	0.6	62 0	0.078
	0.8	40.6	0.082
	1.0	26.4	0.097
	1.2	12.2	0.122

Fig. 12 The effect of BP85 on the chlorophyll content and disease coverage of leaf discs infected with cucumber powdery mildew



Discussion

As can be seen by the value for disease coverage on the unsprayed controls, inoculum level in this experiment was low giving relatively good control at the 1.2 ml level. The disease itself caused a significant reduction in the chlorophyll content of unsprayed discs of about 40%. The oil treatments reduced this until at 1.2 ml the chlorophyll content of sprayed discs was greater than that of the healthy untreated control. Fig. 12 shows the effect of the oil in terms of the percentage disease control and the chlorophyll content of discs expressed as a percentage of the healthy control. The effect of oil on the chlorophyll content increased very rapidly at the higher oil levels suggesting that the oil was delaying the senescence of the discs, this being clearly shown at the 1.2 ml level. A similar effect was noticed by Saunders and Lonnecker (1967) when studying the physiological aspects of using non-phytotoxic oils with herbicides. Corn and Colorado grass were sprayed with oil and together with unsprayed samples were stored in air-tight chambers for 7 d. After this time the oil treated leaves were still green with no outward sign of decomposition whereas the untreated ones had turned brown and decomposition started. No explanation was offered as to the mechanism of action of this effect.

Experiment II

To check the effect of oil on disease and senescence noted above, the experiment was repeated. It was carried out in the college greenhouse and due to the very high temperatures of c. 40°C to 45°C, reached during the experiment, disease development was retarded to such an extent that it could not be clearly measured even after 14 d. Results were thus only obtained for chlorophyll content as given in Table 54.

Table 54 The chlorophyll content of leaf discs sprayed with BP85, inoculated with S. fuliginea and exposed to high temperatures

Treatment	Oil level (ml)	Chlorophyll (O.D. at 665 mμ)
Healthy		
Control	—	0.148
Unsprayed Control	—	0.104
BP85	0.2	0.126
	0.4	0.139
	0.6	0.134
	0.8	0.133
	1.0	0.116
	1.2	0.113

Discussion

The chlorophyll content of discs was significantly lower than the healthy control at all levels except 0.4 ml and in contrast to the previous experiment, decreased with increasing oil level indicating that the oil was phytotoxic under the conditions of the experiment. When expressed as a percentage of the healthy unsprayed control as shown in Fig.12, chlorophyll increased up to the 0.4 ml level and then fell off gradually. Although no values were obtained for disease it would seem likely that the initial increase was due to the almost complete inhibition of the fungus at the low oil levels whilst at higher levels the oil became phytotoxic. The major difference between the two experiments was temperature and it is suggested therefore that

high temperature was responsible for the phytotoxicity of the oil. Some support for this suggestion is given by the work of Havis (1950) who found that although members of the family Umbelliferae appeared relatively immune to hydrocarbons, high concentrations of aromatics and paraffins at high temperatures (38°C) would kill the plants. Dallyn and Sweet (1951), however, asserted that there is no dependence of oil toxicity on temperature among the Umbelliferae.

4. The effect of BP85 exposed to sunlight on cucumber powdery mildew

Brun (1963) suggested that oil sprayed onto banana leaves for the control of Sigatoka leaf spot was gradually oxidized by ultra-violet radiation and became toxic to ascospores of Mycosphaerella musicola. That sunlight renders oil fungitoxic was first shown by Skellern (1968) who exposed BP85 in a clear glass bottle to sunlight for a few weeks. The oil was then tested on glass slides against the germination of Plasmopara viticola which was reduced by 22.2 and 37.5 % at deposit rates of 11.2 and 56.1 mg/100 cm^2. As glass reduces the amount of ultra-violet light passing through it, the inhibition would presumably have been greater if the oil had been exposed directly to the light.

To test whether light treated BP85 was effective against cucumber powdery mildew, a sample of BP85 was left in a clear glass bottle and exposed to sunlight for a few months. This sample was then tested along with the same oil that had been kept in the dark. Discs were sprayed at the 0.4, 0.8 and 1.2 ml oil levels, inoculated and disease assessed at 8 d as usual. Results are shown in Table 55 and Fig. 13.

Table 55 The effect of sunlight on the fungitoxicity of BP85 towards cucumber powdery mildew

Treatment	Oil level (ml)	% Disease cover	% Control
Control	—	100	—

Fig. 13. The effect of sunlight on the toxicity of BP85 towards cucumber powdery mildew

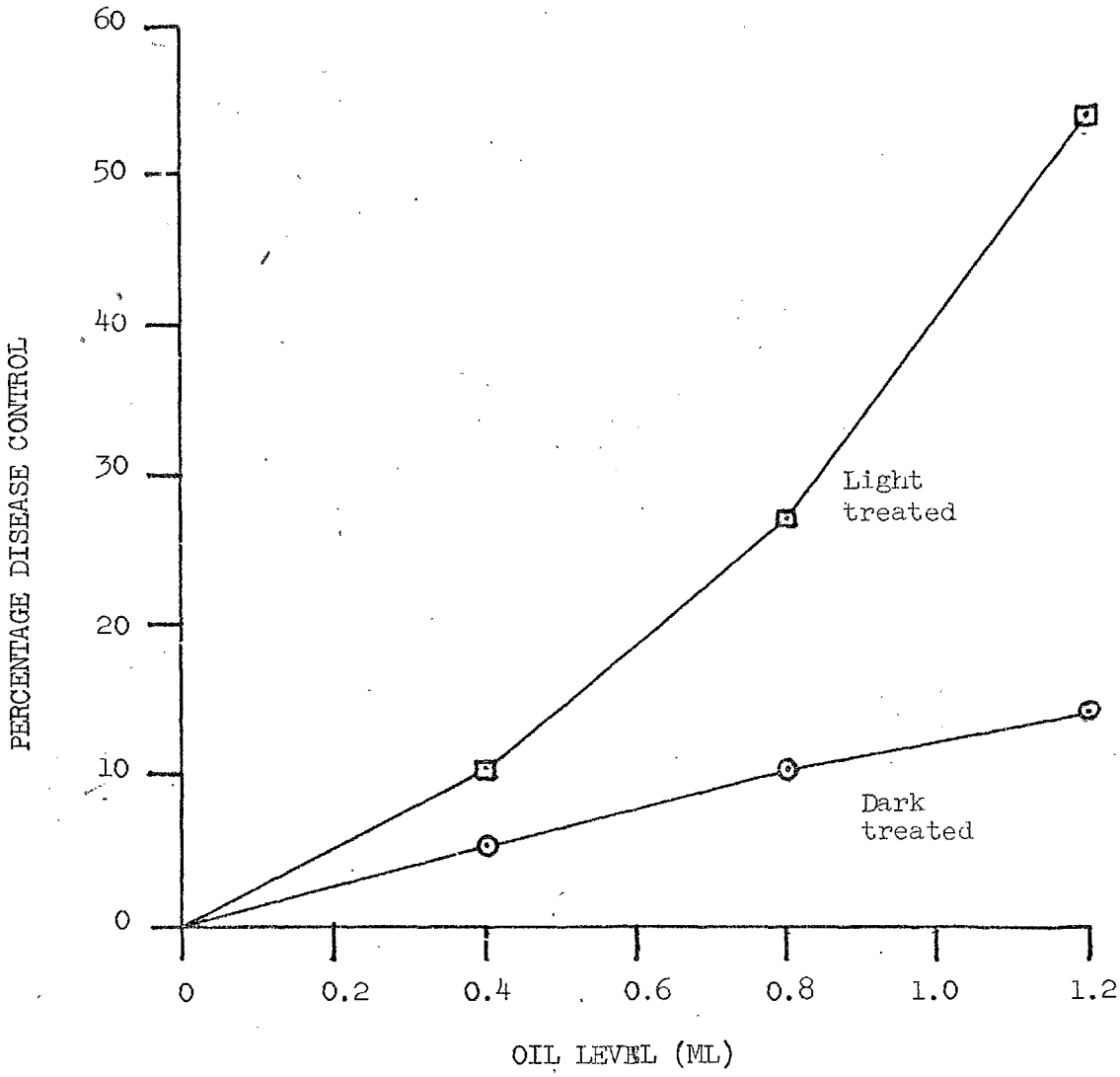


Table 55 cont.

Treatment	Oil level (ml)	% Disease cover	% Control
	0.4	94.8	5.2
BP85	0.8	89.8	10.2
(DARK)	1.2	85.6	14.4
	0.4	89.8	10.2
BP85	0.8	73.0	27.0
(LIGHT)	1.2	46.0	54.0

Discussion

There was a highly significant difference between light and dark treated oils, the light treated oil being more active particularly at the higher oil levels as further shown in Fig.13. This suggests that the light treated oil might have been more active against the mycelium than the dark treated oil, the effect being more noticeable at higher oil levels. Thus, at lower oil levels any inhibitory action on the mycelium would not be very noticeable as the oil did not cover a very large area whereas at the higher levels the leaf area covered would be much greater and therefore the inhibition of mycelial growth more noticeable.

Although the degree of control at the very high deposit rate of 63 mg oil/ 100 cm^2 was only 54%, it is possible that the effect on the disease was produced by a component which was, however, very diluted but which might be extractable and useable as a fungicide on its own.

5. The density of conidial inoculum

Results obtained during the development of the inoculation technique suggested that variations in the degree of control obtained in different

experiments were due to variations in the density of the conidial inoculum. A preliminary experiment was carried out to test this in which the inoculum was varied by using multiples of a standard inoculum source, this being a 17 mm diameter disc cut from a heavily sporulating leaf. Three inoculum levels were used produced by one, two and three discs, the conidia being blown off using a Pasteur pipette and allowed to settle onto leaf discs sprayed at oil levels of 0.4, 0.8 and 1.2 ml as before. Disease coverage was assessed as usual the results being given in Table 56.

Table 56 The effect of variations in inoculum level on disease control - first experiment

Inoculum level (No. of discs)	Oil level (ml)	% Disease Cover	% Control
	0(Control)	98.8	—
	0.4	4.7	95.2
1	0.8	4.5	95.4
	1.2	1.9	98.1
	0(Control)	100	—
	0.4	21.2	78.8
2	0.8	12.4	87.6
	1.2	8.8	91.2
	0(Control)	100	
	0.4	64.0	36.0
3	0.8	24.6	75.4
	1.2	16.2	83.8

Discussion of preliminary experiment

Inoculum level had a considerable effect on disease control. At the lowest level there was very little difference between the three oil levels whilst at higher inoculum levels the lower oil levels were less effective. At the lowest inoculum level a reduction in disease coverage of 95.2% was produced by an oil level of 0.4 ml or 21.3 mg/100 cm^2. Castellani and Matta (1964), however, found almost the same degree of control i.e. 95.4% with the much lower oil level of 8 mg/100 cm^2. This discrepancy between the two sets of results may be explained on the basis of two possible differences between the experiments. First, the oil used by Castellani and Matta may have been inherently more toxic to the fungus than BP85 either by being of a lower boiling point and viscosity as was, for example, gas oil in a previous experiment, or by having deteriorated by exposure to sunlight as previously shown for BP85. Secondly, the inoculum used by these workers may have been lower than that used in the above experiment and it is significant that the conidia were applied as an aqueous suspension, water having been shown to be toxic to the conidia of a number of powdery mildew fungi when they were submerged in it for a prolonged period of time (Zaracovitis, 1964). The inoculation technique of Castellani and Matta may thus have reduced the inoculum concentration of the suspension by reducing the viability of the conidia. The fact that these workers were able to assess the disease in terms of mildew spots, which were therefore presumably isolated, also suggests that the effective inoculum level was very low. In this respect it was found in the present work that with the higher inoculum levels the disease cover could not be assessed in this way, conidia being so close to each other that their branching hyphae became so intermeshed that separate colonies were at no time distinguishable.

To determine the precise relationship between conidial inoculum density and disease control the experiment was repeated using a wider range of inoculum levels measured in terms of the number of conidia/cm^2 using coverslips placed among the leaf discs. The two lowest levels of 96 and 207 conidia/cm^2

were barely visible to the naked eye and produced using the spore release apparatus described previously. Higher levels were produced using whole plants as sources of inoculum and inoculating the discs in a settling tower as described before. The results are given in Table 57 and Fig.14.

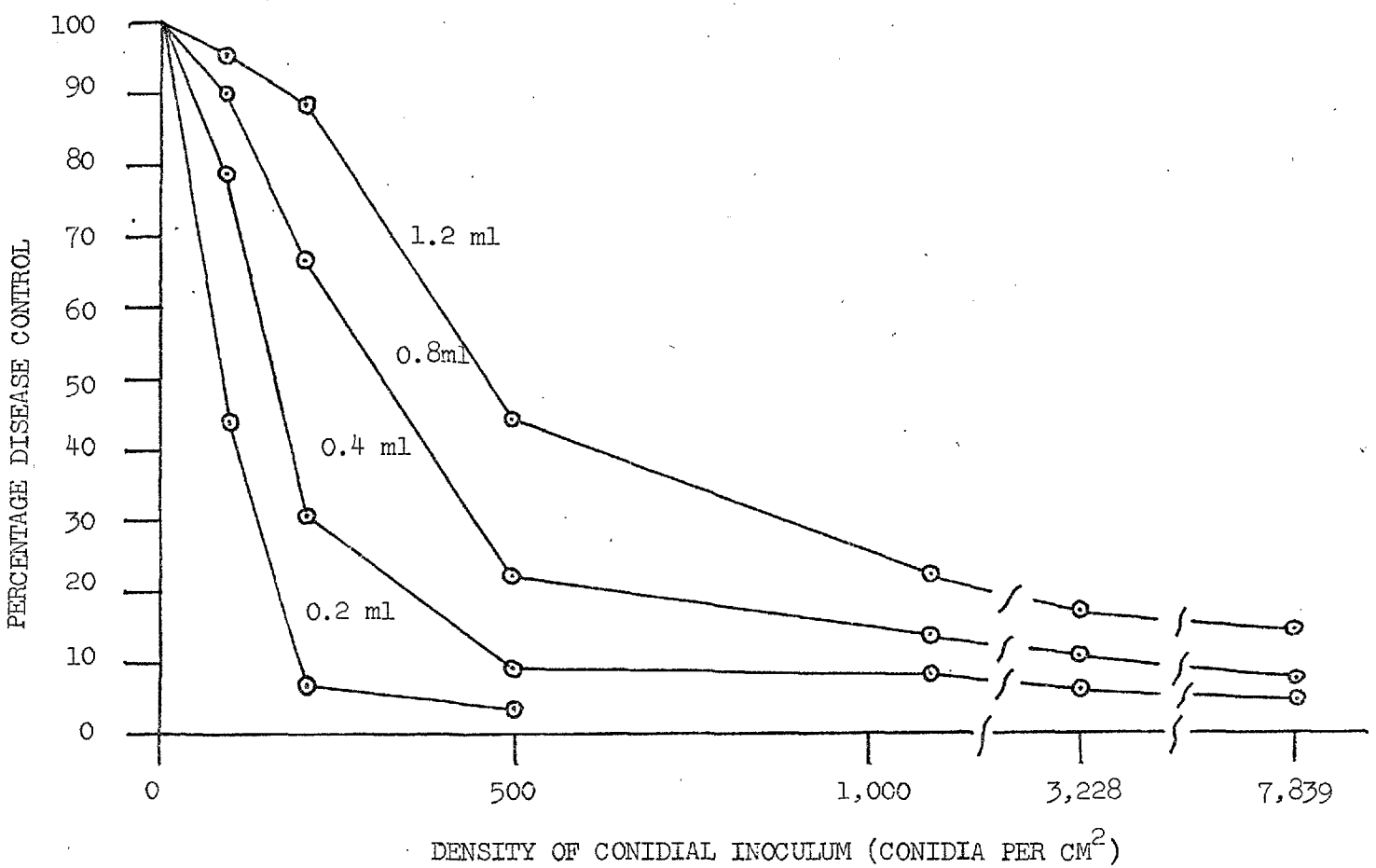
Table 57 The effect of the density of conidial inoculum on disease control - second experiment (% leaf cover)

Inoculum	Oil Level (ml)				
(conidia/cm^2)	0	0.2	0.4	0.8	1.2
96	88.2	50.0	19.0	8.8	4.1
207	99.0	92.6	69.0	33.0	11.6
499	100	96.9	91.0	78.0	56.0
1,086	100	—	92.0	86.4	78.0
3,228	100	—	94.0	89.6	83.0
7,839	100	—	95.5	92.6	85.6

Discussion on the effect of conidial inoculum density

Disease control fell off with increasing inoculum level suggesting that the major effect of oil was on germination with mycelial growth being much less sensitive to the oil. This is supported by Castellani and Matta (1964) who studied the effect of oil on the germination of conidia on cotyledons by taking epidermal strips at intervals up to 3 d. They found that oil prevented conidia from swelling and germinating and that this also applied to germination on water agar. From the results of further experiments they concluded that germination of both quiescent and swelling conidia was inhibited by oil but that after the production of a germ tube they were slightly less sensitive to the oil, the more so after continued growth of the germ tube and

Fig. 14. The effect of the density of conidial inoculum on the control of cucumber powdery mildew



finally that the mycelium was considerably less sensitive again. However, no direct evidence was given for the last three conclusions nor was any work carried out to determine the relative sensitivity of the conidia in their various stages of germination.

Fig. 14 shows a very rapid fall off in control at the 0.2 ml and 0.4 ml oil levels, this rate decreasing with increasing oil level. The greatest fall off for all oil levels occurred at the low end of the inoculum range suggesting a low threshold range for the effect of decreasing inoculum level on germination and disease control. Thus, although the effect of oil on percentage germination would be the same at all inoculum levels, at low levels the actual number of conidia unaffected by the oil would be so small that they would be only capable of colonizing a small area of the disc and thus disease control would be greater than the theoretical area protected by the oil. At higher inoculum levels this number would be correspondingly larger so that the effect of oil on germination would be less significant in that unaffected conidia would be capable of colonizing the whole area of the disc if it were not for the presence of the oil. As the oil would not have a large effect on mycelial growth, disease control would be less than the theoretical area protected by the oil. In the present experiment the threshold range for inoculum level is around 500 conidia/cm^2. Below this range, further decreases have a very large effect on disease control whereas above it, much less so. The width of the threshold range varies for each oil level as can be seen in Fig.14, it being more pronounced and narrow at the lowest oil level, becoming broader as oil level increases. The evidence suggests that the major effect of oil is on germination and thus for any treatment the threshold inoculum level range is defined as the range in which the inhibitory effect of oil on germination becomes significant in determining disease control.

6. A comparison of methods of oil application

In the laboratory oils were normally sprayed using the Potter tower whereas in the field the spinning disc atomizer was used as described previously. The two methods of atomization were therefore compared directly in the laboratory to determine whether there was any difference between the two, in the distribution of oil deposited at the same levels on the control of the disease.

The Micron ULVA was set up in the laboratory as described previously using the litre container as a reservoir for the oil. For spraying, twenty discs were arranged in four Petri-dishes as close as possible to the four sides of a piece of aluminium foil (c.100 cm^2). Four spray times were used viz. 5, 10, 15 and 30 s giving oil deposits of 5.1, 14.1, 20.3 and 46.1 mg/100 cm^2.

The pipette method was used with the Potter tower at levels of from 0.1 ml to 0.9 ml giving deposits as shown in Table 58. Discs were inoculated and disease cover assessed as usual.

Table 58 A comparison of the Potter tower and spinning disc method of oil atomization for mildew control

Spinning disc spray rate	5s	10s	15s	30s
Oil deposit (mg/100 cm^2)	5.1	14.1	20.3	46.1
% Disease cover	95.5	86.4	77.0	56.4
Potter tower spray rate	**0.1ml**	**0.3ml**	**0.6ml**	**0.9ml**
Oil deposit (mg/100 cm^2)	5.5	16.0	31.7	47.5
% Disease cover	96.7	83.0	69.0	59.0

% Disease cover for control = 100

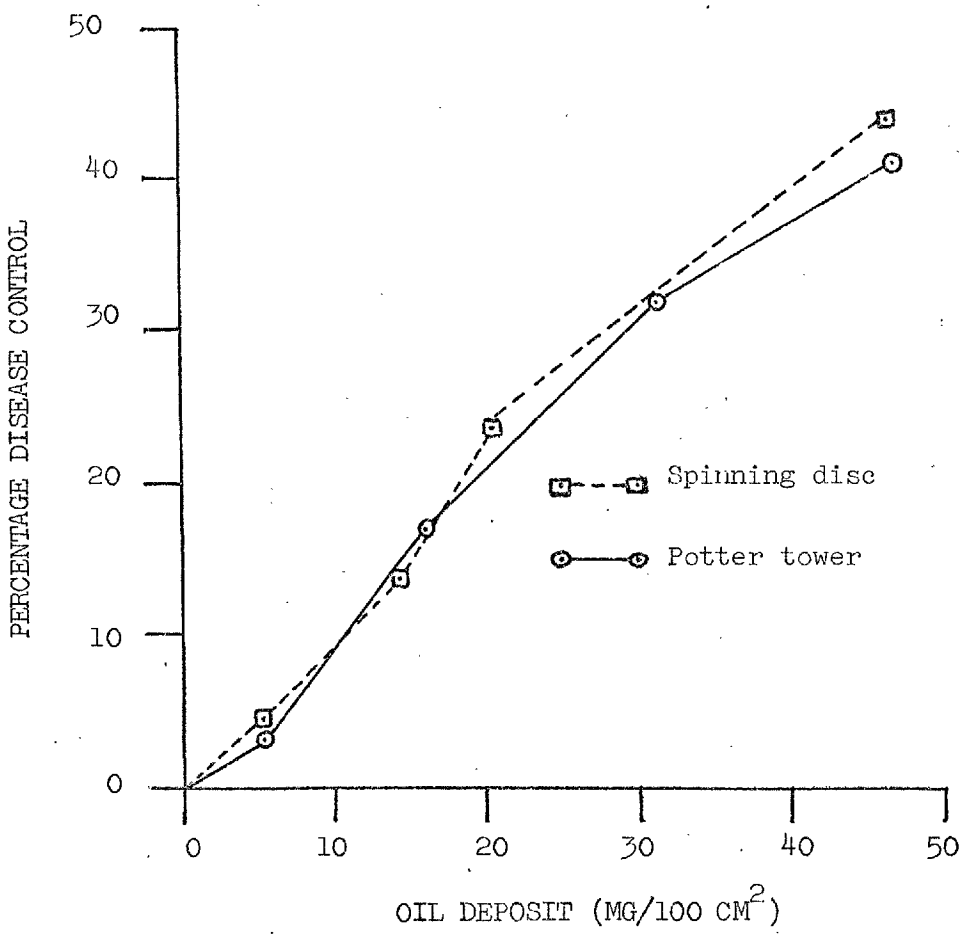
Discussion

As can be seen from Fig.15 there was no significant difference in disease control using the two methods of oil atomization suggesting either that there was no difference in droplet size and/or oil distribution between the two methods or that any difference produced had no measurable effect on disease control.

7. The effect of surface characteristic on oil deposit and disease control

In contrast to the cotyledons, the leaves of cucumber plants are extremely hairy and it was therefore thought likely that the pattern of oil distribution on the two surfaces might be different, so causing a difference in disease control

Fig. 15. A comparison of the Potter Tower and spinning disc methods of atomization of BP85 oil for mildew control



Thus one would expect that a greater amount of oil would be retained on a rough hairy leaf surface than on a smooth, non-hairy surface of the same total area due to the greater friction between the oil-air flow and the rough leaf surface than the smooth cotyledon surfaces. As oil deposits were determined by measuring the deposit produced by the spray on aluminium foil, one would expect the deposit on cotyledons to be closer to this value than that on leaves. The difference between the two might therefore be sufficient to cause a difference in disease control. To test this, discs were cut from both leaves and cotyledons, sprayed at levels of 0.4, 0.8 and 1.2 ml and inoculated as before. The oil deposits on the two surfaces were determined as described previously, and compared with the deposit on aluminium foil. Disease cover was assessed as usual, the results being given in Table 59.

Table 59 Oil deposit and disease control on different surfaces

Oil level (ml)	Oil deposit (mg/100 cm^2)			% Disease control	
	Aluminium foil	Leaf surface	Cotyledon surface	Leaf surface	Cotyledon surface
0.4	13.3	21.7	17.3	4.5	4.2
0.8	31.0	43.5	34.8	11.0	8.4
1.2	47.7	64.6	51.7	17.0	9.0

Discussion

Disease control on the leaf surface was only significantly greater than on cotyledons at the 1.2 ml level. The difference in oil deposit between leaf and cotyledons at this level was significant at the 1% level whereas at the 0.8 ml level it was only significant at the 5% level. Thus although the percentage increase in oil deposit on leaf surfaces as compared with cotyledon surfaces was the same at all levels(i.e. c. 25%), the actual increase of 12.9 mg/100 cm^2 at the 1.2 ml level was sufficiently large to cause a

significant increase in disease control whereas the increase of 8.7 mg/100 cm^2 at the 0.8 ml level was not.

The oil deposit on the leaf was significantly greater than on aluminium foil at all oil levels whereas there was no significant difference between the deposit on foil and cotyledons.

8. The persistence of BP85

Whilst previous experiments had shown that disease control with BP85 was poor except at low inoculum levels, it was thought possible that this might be improved by allowing the oil to remain on the plant for prolonged periods of time before inoculation. This might be brought about by either an indirect action via the host as suggested for its effect on vine downy mildew in Section I or by its spread over a large area of leaf surface than initially covered by the spray.

Discs were cut and sprayed at 8, 6, 4 and 1 d before and immediately after inoculation. Disease cover was estimated as before. Results are shown in Table 60.

Table 60 The effect of pre-inoculation sprays of BP85 on percentage disease control

Oil level	Pre-treatment time (d)				
(ml)	8	6	4	1	0
0.4	5.6	6.0	6.0	4.0	1
0.8	19.2	19.2	14.4	11.8	7.4
1.2	31.0		25.2	20.8	20.8

Discussion

The effect of pre-inoculation spraying was only significant at the lower oil levels though in these cases, control was only slightly improved. At the 1.2 ml oil level control increased by 10% but this was not sufficiently

large to be significant due to the width of the grades used to measure the disease in this case. At the lower oil levels, disease cover was greater and therefore amongst the narrow grades at the end of the Horsfall-Barratt scale so that smaller differences were significant.

9. The eradicant action of BP85

Previous experiments suggested that the major effect of BP85 on the fungus was the inhibition of germination. Thus one would expect its activity to fall off very rapidly when sprayed after inoculation. To test this, discs were inoculated and either sprayed immediately or at day intervals up to 7 d after inoculation. Disease cover was assessed as before and sporulation on the 0.4 ml treated discs estimated in terms of the number of conidia/cm^2 released from the discs using the spore release apparatus and method described previously. Results are shown in Tables 61 and 62.

Table 61 The effect of post-inoculation sprays of BP85 on percentage disease control

Oil level	Post-inoculation spray time (d)							
(ml)	7	6	5	4	3	2	1	0
0.4	0	0	1	1	2.4	4.5	4.0	3.1
0.8	0	0	1	1.0	3.1	4.0	6.8	6.4
1.2	0	0	1.9	4.2	4.0	9.0	9.0	11.0

Table 62 The effect of post-inoculation sprays of BP85 (0.4 ml) on sporulation (conidia released)

Days	Control	7	6	5	4	3	2	1	0
Conidia/ cm^2	807	459	346	165	53	8	1	0	0

Discussion

As before, disease control was poor, falling off to zero on discs treated 5 or 6 d after inoculation. The effect on sporulation, however, was quite marked, the number of conidia produced on 0 and 1 d treated discs, being so small as to remain undetected using the spore release method. Even when treated 7 d after inoculation, sporulation was still reduced by about 45%. Thus it would seem that whereas mycelial growth was not greatly reduced by oil, sporulation, at least on leaf discs, was strongly inhibited. This aspect was not studied by Castellani and Matta (1964) who merely showed that a delay in oil application after inoculation caused an increase in mildew patches, as detected with a hand lens, on treated leaves.

10. The effect of oil on the release of conidia from sporulating leaf discs

Due to the inherent hydrophobic nature of both oil and mildew conidia it was thought likely that oil sprayed onto sporulating cultures of mildew on leaves would cause the conidia to adhere to each other and to the conidiophores thus preventing their release by air currents. This would be of particular importance as this is the normal method of dispersal of the fungus.

a) Development of the technique

The apparatus described in Materials and Methods, part B, 3 b) was designed to simulate the wind dispersal of conidia, standardizing as many variables as possible. The repeatability of the technique is illustrated in Table 63 which shows the deposits of conidia blown off with a Pasteur pipette from five sets of discs cut from the same sporulating leaf material.

Table 63 The repeatability of the spore release technique

Replicate	conidia/cm^2
1	1,105
2	1,155
3	1,163
4	1,081
5	1,064

As the major variable in the release of conidia from leaves would be the strength of the wind, various strengths or speeds were simulated by holding the pipette at varying distances from the leaf discs. The effect of this is clearly shown by the two examples in Table 64.

Table 64 The effect of air speed (distance from pipette to discs) on the release of conidia

Distance from pipette to discs	Conidial release (conidia/cm^2)
1.0 cm	2,095
2.0 cm	1,070

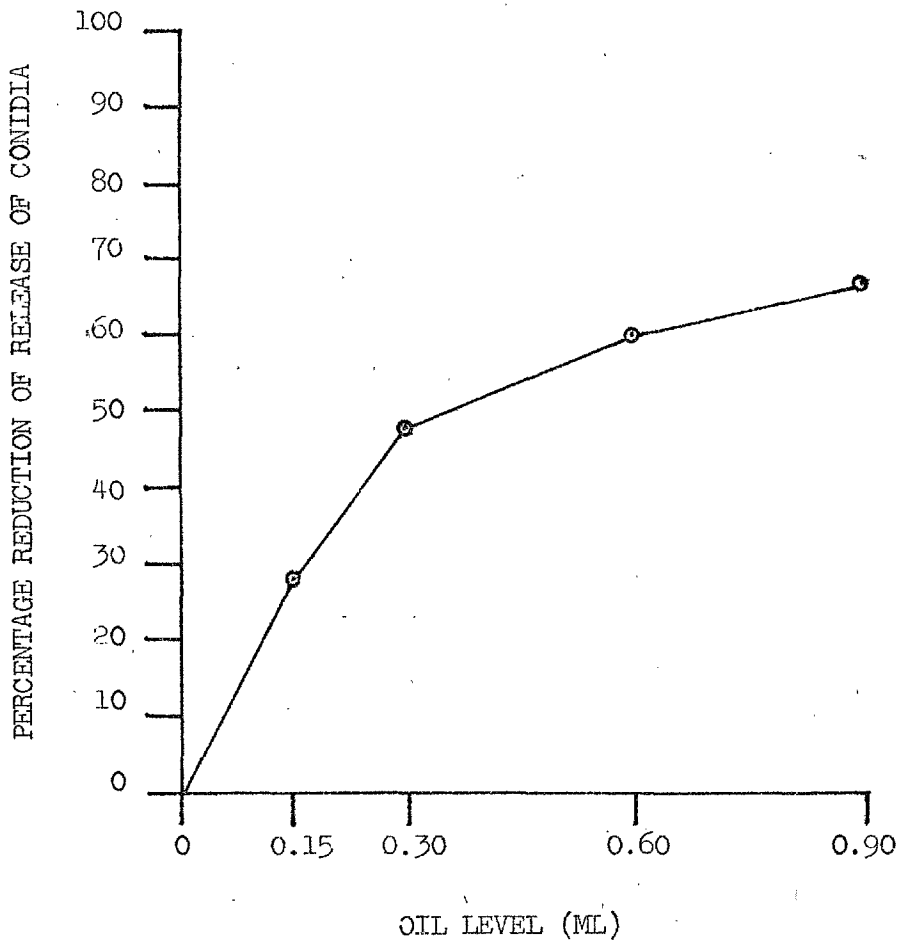
b) The effect of oil on conidial release

Sporulating discs were sprayed with BP85 at levels from 0.15 ml to 0.90 ml using the pipette method with the Potter tower and giving the results shown in Fig.16.

Discussion

The oil caused a highly significant reduction in the release of conidia, the effect levelling off at around 65% at the 0.9 ml oil level.

Fig. 16. The effect of BP85 on the release of conida from sporulating leaf discs



c) The effect of varying air speeds (distances from pipette to discs) on the reduction in release of conidia

As wind speed varies greatly in the field it was thought possible that this might affect the magnitude of the reduction in conidial release. Thus at low wind speeds it might be expected that only those conidia unaffected by oil would be released, whereas at high speeds both these, and also those embedded in the oil, would be released so producing a smaller reduction in conidial release than at the lower air speed. To test this, three air speeds were simulated using three distances between pipette and leaf discs. Discs were sprayed at the 0.15 ml oil level. Results are shown in Table 65.

Table 65 The effect of air speed on the reduction in conidial release

Air speed (distance from pipette to discs)	Conidia/cm^2		% Reduction of Conidial release
	Control	Oil	
1.5 cm	2,211	1,579	28.6
3.0 cm	1,303	929	28.7
4.5 cm	958	690	28.0

Discussion

Variations in simulated air speed had no significant effect on the magnitude of the reduction in release of conidia and it was noticed that even at low air speeds, oil affected conidia were released. Thus in this experiment a threshold air speed was not found, below which the oil affected conidia were not released.

C. The Effect of BP85 on Sphaerotheca fuliginea

As the fungus is an obligate parasite it was only possible to test the effect of oil on germ tube and mycelial growth in vivo whereas the effect of oil on germination was tested both in vitro and in vivo.

1. Germination and production and growth of hyphae in vivo

Castellani and Matta (1964) showed that a light mineral oil completely inhibited the germination of conidia of E. cichoracearum both on the surface of cotyledons and on water agar. They further showed that when sprayed onto young cucumber plants at a rate of 8 mg/100 cm^2 immediately after inoculation or 1, 2, 3 and 4 d later, disease control assessed at 7 d was respectively 100, 100, 95.5, 92.7 and 72.7 %. At these times they stated that the fungus was respectively in the form of quiescent conidia, swollen conidia, germinating conidia, branched germ tube and intertwined hyphae and therefore made the following conclusions:-

1) both quiescent and swelling conidia were equally sensitive to oil;
2) their sensitivity to oil was slightly reduced after germination and appressorium formation and more so after further growth of the germ tubes;
3) the mycelium was considerably less sensitive to oil although its development was clearly retarded and in some cases completely inhibited.

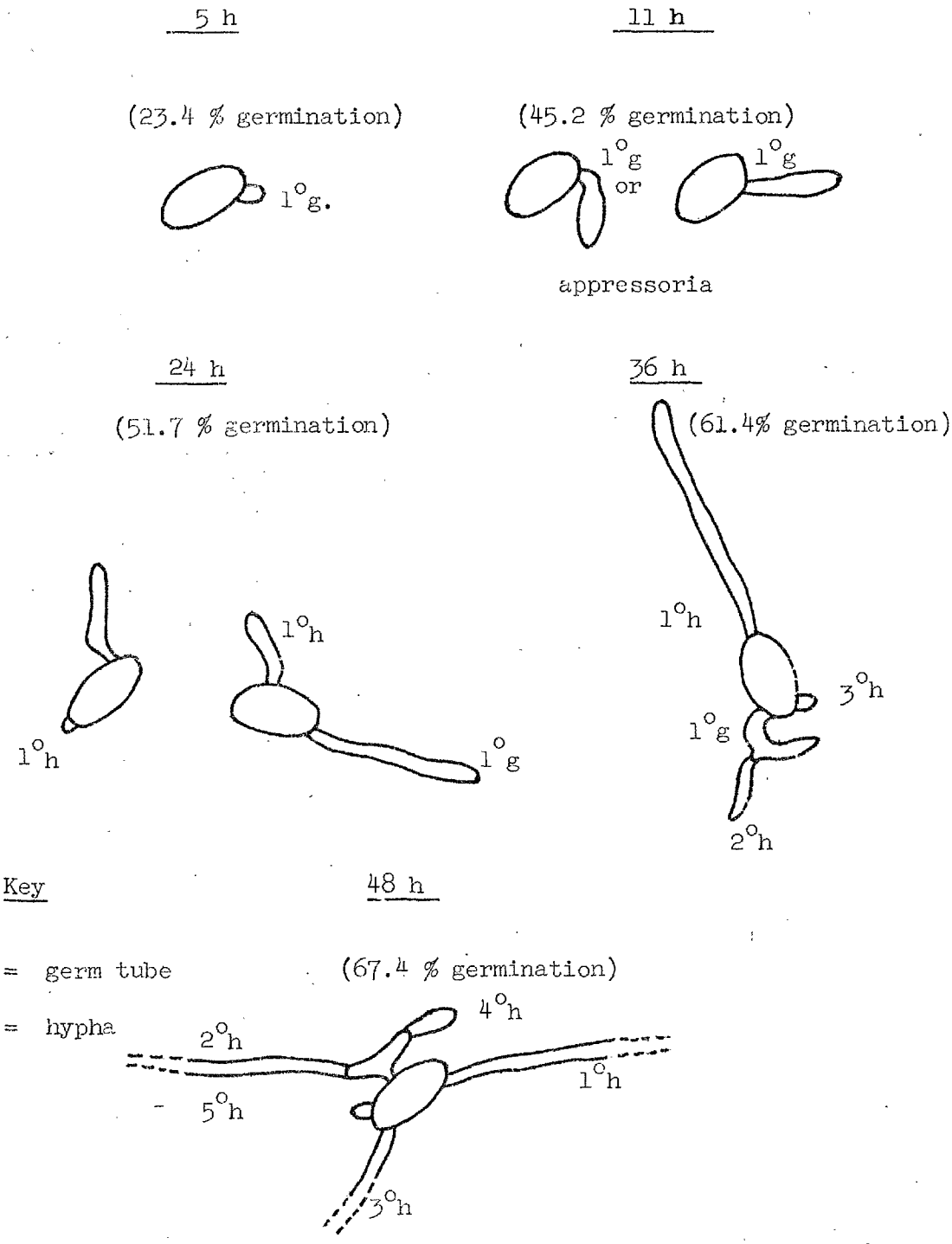
In order to determine whether BP85 acted in the same way in the present experiment, leaf discs inoculated with conidia of S. fuliginea were sprayed with 63 mg oil/100 cm^2 (1.2 ml oil level) immediately after inoculation or 5, 11, 24, 36, 48, 80 and 96 h later. The celloidin peel technique described previously was used to determine the stage of development of the fungus at these times and germination, production and growth of hyphae at 48 h and disease cover at 8 d.

a) Development stages of the fungus

The stages of development of the fungus at the above times were as follows and as illustrated in Fig.17.

1) 5 hours. Germ tubes were just forming, germination being 23.4 %, and the length of the germ tubes about $\frac{1}{4}$ to $1\frac{1}{4}$ the width of the conidia.

FIG. 17 Time sequence for the stages of development of Sphaerotheca Fuliginea



2) 11 hours. Germination had increased to 45.2 % with the germ tubes about twice as long as at 5 h. Appressoria had formed and were typically bent as shown in Fig. 17.

3) 24 hours. With germination at 51.7 %, primary hyphae (1^{o}h.) were formed from the end opposite the germ tube.

4) 36 hours. Germination had increased to 61.4 % with the majority of the germinated conidia at the stage illustrated in Fig.17. A secondary hypha (2^{o}h.) had formed from the appressorium, the 1^{o} hypha increasing in length and a tertiary hypha (3^{o}h.) formed from the opposite end to the primary hypha.

5) 48 hours. By this time germination was 67.4 % with primary and secondary hyphae continuing to grow and tertiary hyphae also increasing in length. In some cases a fourth hypha (4^{o}h.) had been produced from the appressorium, a fifth hyphae (5^{o}h.) from the germ tube end of the spore and the primary hypha had begun to branch.

6) 80 hours. By this time the hyphae had become so long that it was difficult to distinguish where they originated from.

b) The effect of BP85 on germ tube growth

The effect of oil applied at 0 h on the length of 1^{o}, 2^{o} and 3^{o} hyphae at 48 h is given in Table 66.

Table 66 The effect of BP85 on germ tube growth (eye piece units)

Hyphal type	Control	Oil	% growth reduction
1^{o}h.	4.2	3.0	28.6
2^{o}h.	2.4	1.9	20.8
3^{o}h.	1.3	1.1	15.4

Growth was significantly reduced in all three cases, the reduction being greater for the longer hyphae suggesting that growth over a certain distance was

presumably necessary before the germ tubes could come into contact with the oil and therefore be retarded.

c) The effect of BP85 on germination and germ tube production

The effect of the oil on the fungus was determined both with respect to its effect on germination and production of hyphae. The results are given in Figs. 18 and 19 with hyphal production expressed as a percentage of the total conidia and as a percentage of the germinated conidia respectively. Table 67 shows germination of conidia at the 5, 11, 24, 36 and 48 h spray times with germination of conidia at 48 h on discs sprayed at these times.

Table 67 The effect on germination at 48 h of BP85 sprayed at 0, 5, 11, 24 and 36 h after inoculation

Spray time (h)	% germination (controls)	% germination at 48 hours (oil sprayed)	% reduction in germination caused by oil
0	0	32.8	51.3
5	23.4	57.5	14.7
11	45.2	63.9	5.3
24	51.7	65.5	2.8
36	61.4	66.7	1.0
48	67.4	—	—

Discussion

The effect of oil on germination fell off very quickly when the oil was sprayed at increasingly long periods after inoculation. This fall off follows a simple mathematical relationship between the percentage reduction in germination caused by the oil sprayed at 0 h (i.e. 51.3 %) and the rapidly decreasing number of conidia that are left to germinate after the oil has been sprayed. Thus after 5 h, 23.4 % conidia had germinated leaving 44.0 %

Fig. 18 The effect of BP85 sprayed at 0, 5, 11, 24 and 36 hours after inoculation on hyphal production (% of total conidia)

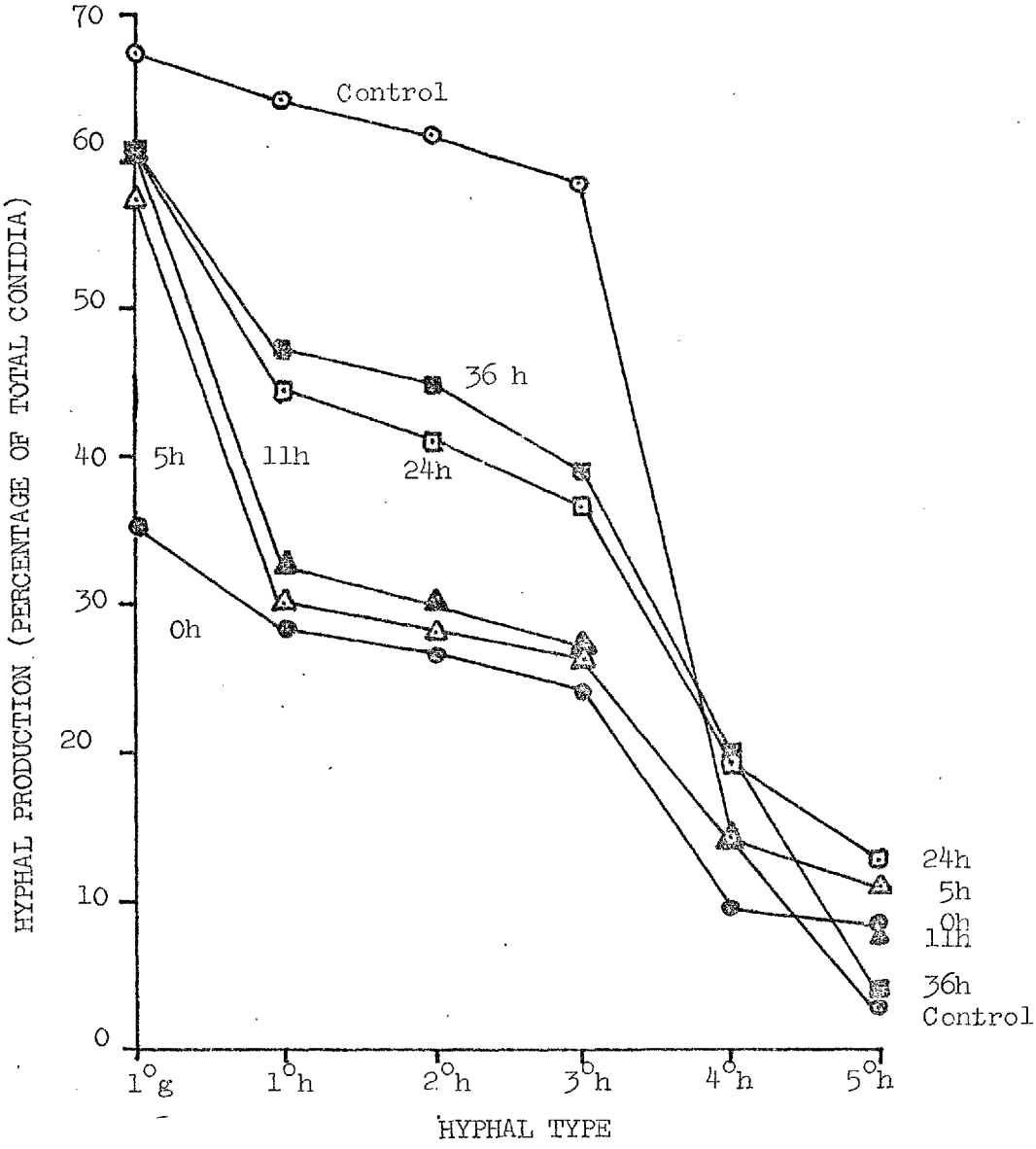
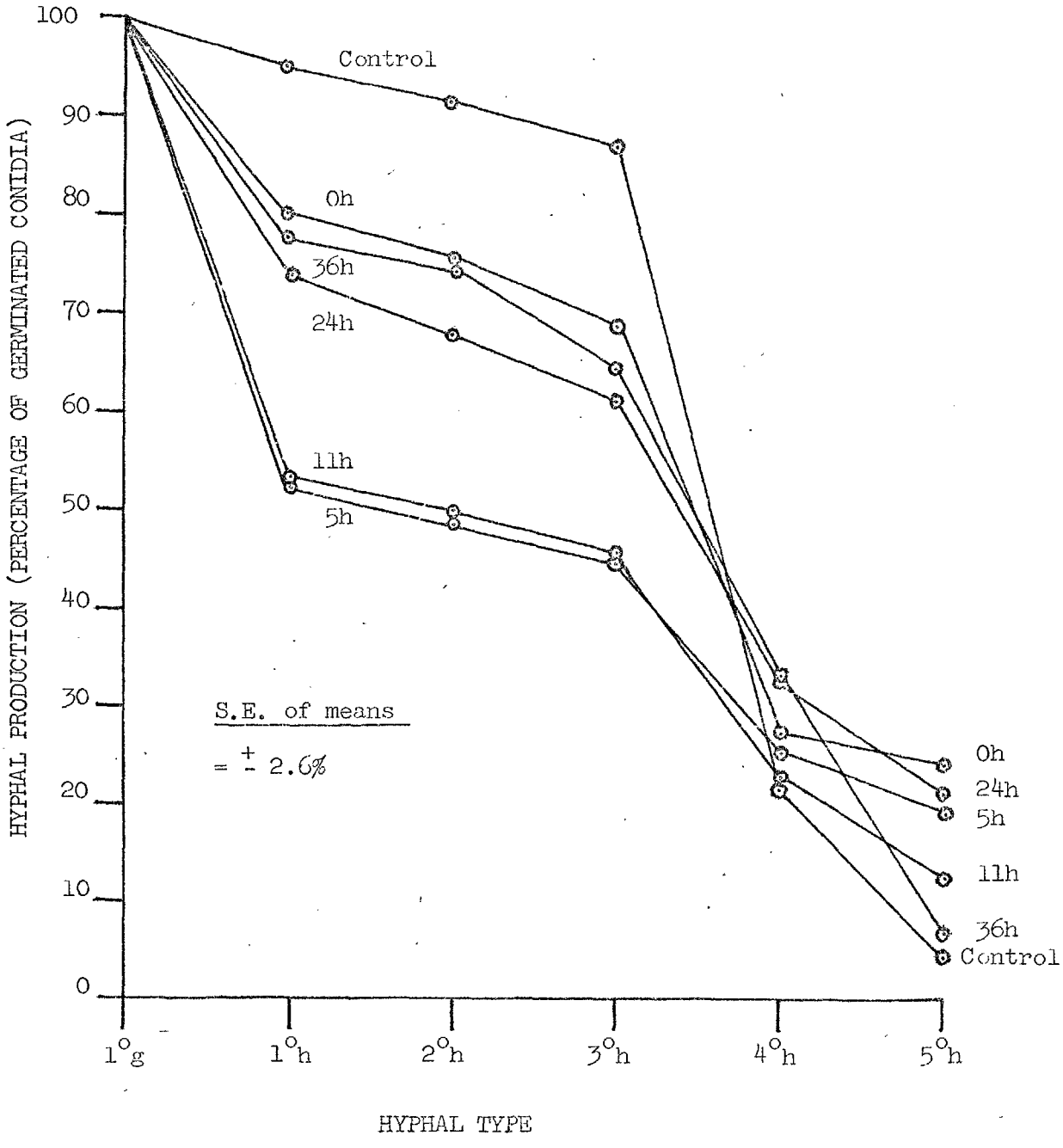


Fig. 19 The effect of BP85 sprayed at 0, 5, 11, 24, and 36 hours after inoculation on hyphal production (% of Germinated Conidia)



of the total to germinate between 5 h and 48 h and thus be affected by the oil sprayed at 5 h. The effect of the oil on this 44 % was then not 51.3 % but 22.8 %. Similarly, oil sprayed at 11 h only caused an 11.4 % reduction of only 22.2 % potentially germinable conidia.

The number of conidia with 1^{o}, 2^{o} and 3^{o} hyphae was very much retarded by the 5 and 11 h treatments which also differed significantly from the smaller reduction caused by the 24 and 36 h treatments. The production of 2^{o} hyphae and 4^{o} germ tubes was significantly stimulated as indicated by the standard errors of Fig. 19. As the percentage germination caused by the 0 h treatment was very much reduced, the effect on germ tube production differed in the two figures. Thus Fig. 18 indicates the effect of the oil on the ability of the total population of conidia to produce germ tubes and hyphae whereas Fig. 19 shows the effect of oil on the ability of those conidia which germinate to produce germ tubes and hyphae. When oil was sprayed at 0 h, Fig.18 shows there to have been a quite large effect on the total conidia at 48 h with 1^{o}, 2^{o} and 3^{o} hyphae. Fig. 19, however, shows that of those conidia germinated at 48 h, the 0 h treatment had only a small effect on hypha production.

Fig. 19 allows some tentative conclusions about the effect of oil on the fungus from the results of the effect of oil on the production of hyphae by germinating conidia. It is suggested, therefore, that the oil affects the fungus via:-

a) the conidium by (i) inhibiting germination when it is completely embedded in the oil and (ii) slowing down the growth and production of new hyphae when the conidium is only just touching the oil or is not completely embedded in the oil. This effect would be greater when the spore was at an early stage of hyphal development than when at a later stage. A similar effect might be produced by the conidium at a later stage when it was less sensitive to the oil but when it was completely embedded in the oil.

It is also suggested that the oil affects the fungus via:-

b) the hyphae by slowing down their growth and possibly as a consequence, stimulating new hyphal production.

The oil would therefore produce two populations of germinated conidia. Population I would be slow developing conidia (effect (a) (ii)) with the production of hyphae very much slowed down. Population II would be rapidly growing germinated spores in which the oil would reduce and/or so affect the growth of the hyphae as to stimulate the production of new ones.

On the basis of these two populations the following is a suggested explanation for the effect of oil when sprayed at the above mentioned times after inoculation. When applied at 0 h the oil has very little effect on hypha producing conidia as these spores embedded in the oil do not germinate and remain in this state. The c. 16 % reduction in hypha production up to and including the 3° hypha is produced by the population I conidia. The slight stimulation of 4° hyphae and very significant stimulation of 5° hyphae is produced by population II. By 5 h, c. 23 % conidia have germinated and may therefore be affected by the oil. Thus, these conidia contribute to the population I effect whilst the population II effect is reduced. By 11 h germination has reached c. 45 % with the extra germinated spores contributing to the population I effect when sprayed with oil. The 23% of conidia which formed the population I effect at the 5 h application are now less sensitive to oil and their contribution to the population I effect up to the 3° hypha is therefore very small. Thus the total population I effect is not significantly greater than with the 5 h treatment. The population II effect is, however, again reduced, causing a reduction in the stimulation of 5° hypha production. By 24 h germination has only increased by c. 7 % and thus the population I effect produced by these is very small whilst the conidia which produced the population I effect with the 5 and 11 h treatments are less sensitive to oil. Thus the total population I effect is reduced being more

close to that caused by the 0 h treatment. When oil is applied at 24 h 1^{o} hyphae have already been produced by conidia. These do not grow far before coming into contact with oil and thus 4^{o} and 5^{o} hyphae are produced at a faster rate than at 11 h due to the population II effect. By 36 h the conidia have formed 3^{o} hyphae and thus there is not suggicient time left between 36 and 48 h for the oil to sufficiently affect these hyphae to cause any stimulation in the production of 5^{o} hyphae though sufficient time is left for the stimulation of 4^{o} hyphae.

d) The effect of BP85 sprayed after inoculation on disease cover

Discs inoculated and sprayed in the above experiment were allowed to progress to sporulation when disease cover was assessed as usual. Results are given in Table 68.

Table 68 The effect of spray time on disease control

Spray time (h)	Control	0	5	11	24	36	48	80	96
% Disease cover	100	92.1	92.7	92.1	93.2	94.5	95.9	95.5	98.7
% Disease control	—	7.9	7.3	7.9	6.8	5.5	4.1	4.5	1.3

Discussion

Although disease control was very poor the pattern of the effect of spray time was similar to that obtained by Castellani and Matta (1964) described earlier. Thus these workers found no difference between spraying at 0 or 1 d intervals, a significant difference between 1 and 2 d, a small difference between 2 and 3 d and a large difference between 3 and 4 d. In the present experiment also, significant differences were only found between 1 and 2 d and between 3 and 4 d. Whereas, however, Castellani and Matta stated that at 24 h, conidia were swollen but ungerminated, in the present experiment germination was nearly complete at this time. Furthermore, although Castellani and Matta concluded that because disease control was considerably reduced when oil was sprayed at 4 d rather than at 3 d (72.7 % control compared with 92.7 %) this

indicated that the mycelium was considerably less sensitive to the oil, the fungus at this time was itself very well developed. It was not, however, stated whether the fungus at this stage of development (intertwined hyphae) would be disregarded when counting Oidium patches using a magnifying lens. In the present experiment germination at 48 h was reduced by the oil treatment at 0 h by 51.3% whereas the 2° germ tubes were reduced in growth (length) by only 28.6 %. This does provide some quantitative evidence that the mycelium is less sensitive to oil than the conidia. The results for the effect of oil on disease control do not, however, provide any strong evidence that a germinated conidium is less sensitive than a quiescent or ungerminated swollen conidium as suggested by Castellani and Matta.

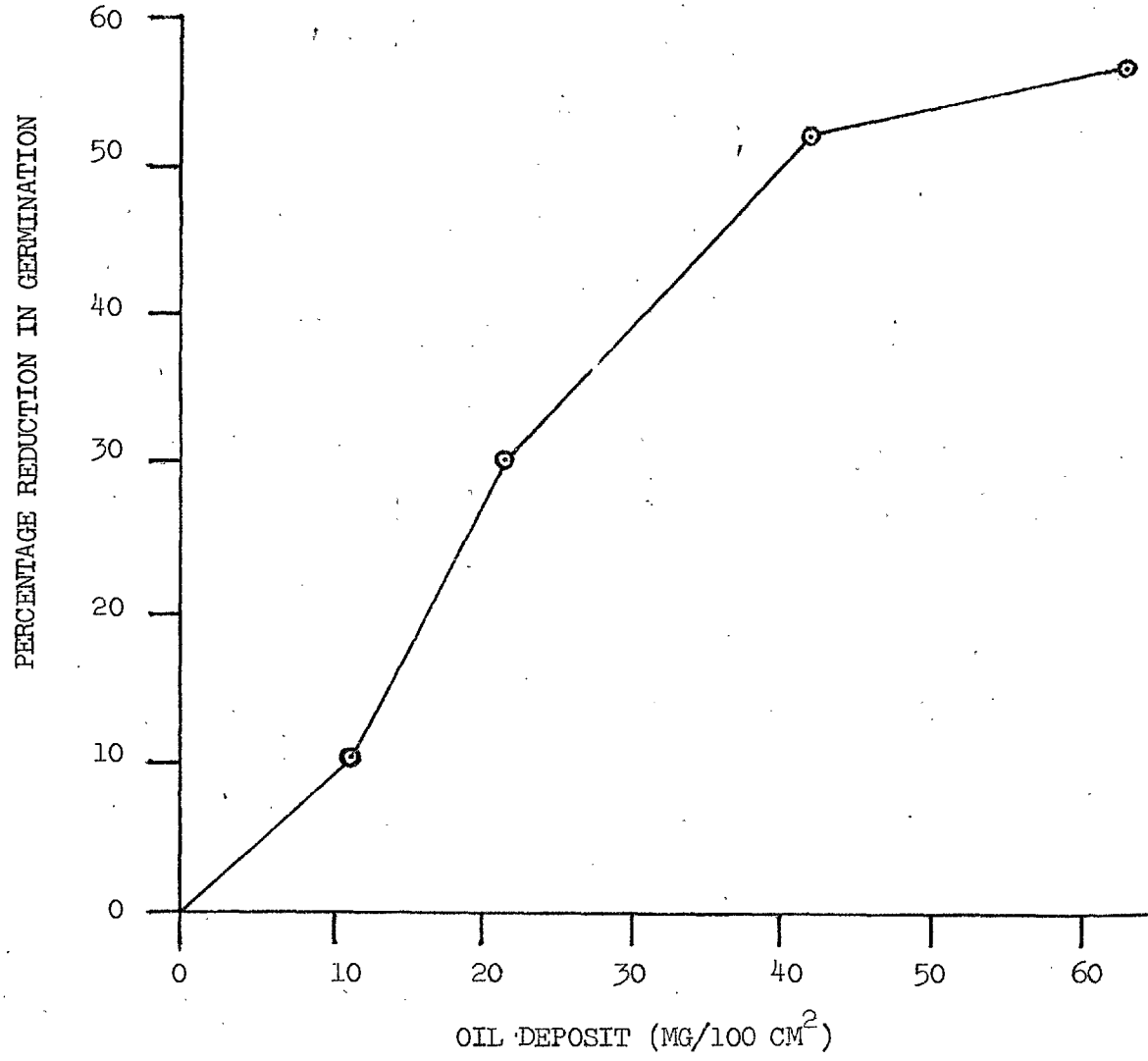
2. The effect of BP85 on germination in vitro

Clean coverslips were sprayed at levels of 0.2, 0.4, 0.8 and 1.2 ml of BP85 and conidia deposited on them and incubated in Manners and Hossain (1963) germination chambers as described previously. Germination was assessed after 48 h. The results expressed as percentage reductions in germination are given in Fig. 20.

Discussion

All treatments were significantly different from the control with the percentage reduction of germination falling off at the higher oil levels. Thus very high levels would be required to give complete inhibition of germination. Percentage reduction was higher in this experiment on glass than in the previous experiment on the leaf (56.7 % compared with 51.3 %). This difference may reflect the difference in spreading of the oil on glass and plant surface. This was suggested by Skellern (1968) as a reason for the coalescence of droplets of oil sprayed onto glass slides. She suggested that coalescence would be less likely to happen on leaves due to the uneveness of the surface. The fall

Fig. 20 The effect of BP85 on the germination of conidia of *Sphaerotheca fuliginea* on glass



off in percentage reduction of germination on glass was expected due to the non linear relationship between oil deposit and area so covered by the oil. Thus it would be expected that at increasingly higher levels a greater percentage of the oil sprayed would deposit on areas already covered with oil without causing a corresponding increase in unaffected area covered with oil.

The results of both experiments on germination in vitro and in vivo show that as germination is only reduced by about 50 to 60% the number of conidia left to germinate will be considerable except at low inoculum levels.

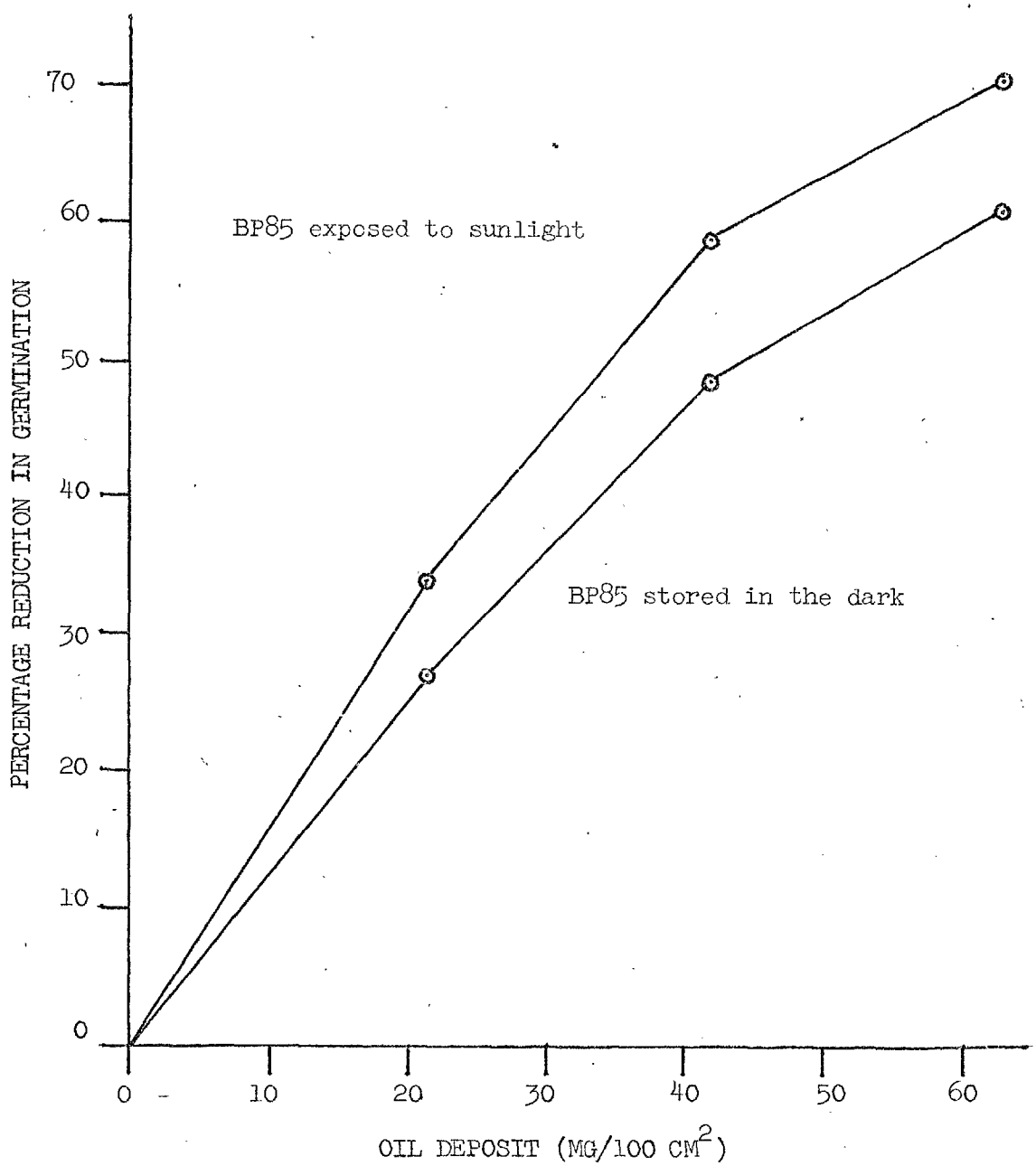
3. The effect of sunlight on the in vitro toxicity of BP85

A previous experiment showed that oil exposed to sunlight was much more effective in controlling cucumber powdery mildew than oil stored in the dark. The same two oils were therefore compared against the in vitro germination of the fungus, Sphaerotheca fuliginea. Coverslips were sprayed, dusted with conidia, incubated and assessed as in the previous experiment. The results, expressed as percentage reductions in germination of the control are given in Fig. 21.

Discussion

The significant increase in toxicity of BP85 at the two higher levels of 42 and 63 mg/100 cm^2 when exposed to sunlight, on the germination of S. fuliginea was relatively small when compared with the increased activity of this oil on the disease shown previously. This suggests that BP85 stored in the dark is not very effective against the mycelium of the fungus in vivo but when exposed to sunlight becomes much more inhibitory to it. It also supports a previous suggestion that many conidia, though in contact with the oil are not inhibited. Thus in the present experiment, conidia which were not completely embedded in the oil and would therefore not normally be inhibited from germinating, are completely inhibited by the oil exposed to sunlight.

Fig. 21. The effect of sunlight on the toxicity of BP85 to Sphaerotheca fuliginea



D. The Effect of BP85 on Cucumber Powdery Mildew using Whole Plants

Experiment I.

BP85 was sprayed onto plants at the four leaf stage using the Micron ULVA as described before and with the oil deposits determined using aluminium foil. Plants were inoculated in a large settling tower as usual and disease assessed on those leaves which were fully expanded and not shaded by others at the time of spraying and inoculation. The results are given in Table 69.

Table 69 The effect of BP85 on cucumber powdery mildew using whole plants

Oil deposit (mg/100 cm^2)	0	1.9	14.7	36.9	83.8
% Disease cover	100	99.7	81.5	35.3	5.5
% Control	—	0.3	17.5	64.7	94.5

Discussion

Although no direct comparison could be made between this experiment and those using leaf discs as the inoculum level was not determined, it was clear that a very high level of oil was necessary to control the disease. Plate 8 illustrates representative leaves taken from the experiment with the oil levels of 5, 15, 30 and 60 s corresponding to the deposit rates given in Table 69. The inoculum level used was much higher than one would normally expect in the field at the beginning of an epidemic. Thus BP85 might prove effective at the beginning of a season whereas later on as the inoculum level in the field built up it would become completely ineffective at commercially acceptable rates of usage i.e. below 10 mg/100 cm^2.

E. The Phytotoxicity of BP85 on Whole Plants

Although BP85 was ineffective against mildew, except at low inoculum levels, the oil was still of use as a carrier for conventional fungicides. Thus any phytotoxic effects caused by the oil would be important.

1. The effect of BP85 on plant growth

Internode lengths of plants sprayed with oil, using the Micron ULVA, were



PLATE 8 Effect of EP85, sprayed with the Micron ULVA for 0, 5, 15, 30 and 60 sec. on cucumber powdery mildew (c = unsprayed, uninoculated control).

measured after 20 d, in a greenhouse at c. 20°C to 26°C. The average total plant growth of six replicates sprayed with 0, 8.0 and 94.8 mg/100 cm^2 was, respectively 20.0, 21.2 and 13.2 cm . Thus the high level of oil significantly reduced plant growth by 34%. Considering the heaviness of the deposit (more than ten times an acceptable commercial level, this was only a small effect and was not accompanied by any other signs of phytotoxicity such as oil soaking.

2. The effect of BP85 on leaf permeability

The effect of BP85 on the leakage of ions from cucumber leaves was taken as a measure of microscopic injury. Plants were sprayed with the Micron ULVA as before except that one half of each leaf was covered with a polythene bag sealed to the mid rib. As only four plants could be accommodated in the spray chamber, a replicate for each of the four sample times was used with each of four sprays. The four deposits of oil were very close to each other being 74.2, 74.3, 76.2 and 71.0 mg/100 cm^2 as measured using aluminium foil in the centre of the four plants each time. Treated plants were left in the greenhouse and sampled immediately after spraying and 5, 12 and 19 d later and the leakage of ions from the leaves measured as described previously. Leakage was measured at intervals up to 48 h, the change in leakage with leaching time after 19 d being given in Fig.22 and the change in leakage with time after spraying given in Fig.23. In both, leakage was expressed as a percentage of the total electrolyte in the leaf samples determined by boiling them.

Discussion

BP85 applied at the high level of 73.8 mg/100 cm^2 caused significant membrane damage as measured by ion leakage at 12 and 19 d. The relatively large increase in leakage over the control of 76.9 % suggests that at lower levels a smaller though similar type of damage might occur indicating oil phytotoxicity which might not otherwise be detected except perhaps in a decrease in yield. The leakage pattern for both leaching and treatment time illustrates the time required by relatively viscous oils such as BP85 to penetrate the leaf as compared with a very light oil such as n-decane used

Fig. 22. The change in ion leakage from control and BP85 sprayed leaf samples with leaching time at 19 days

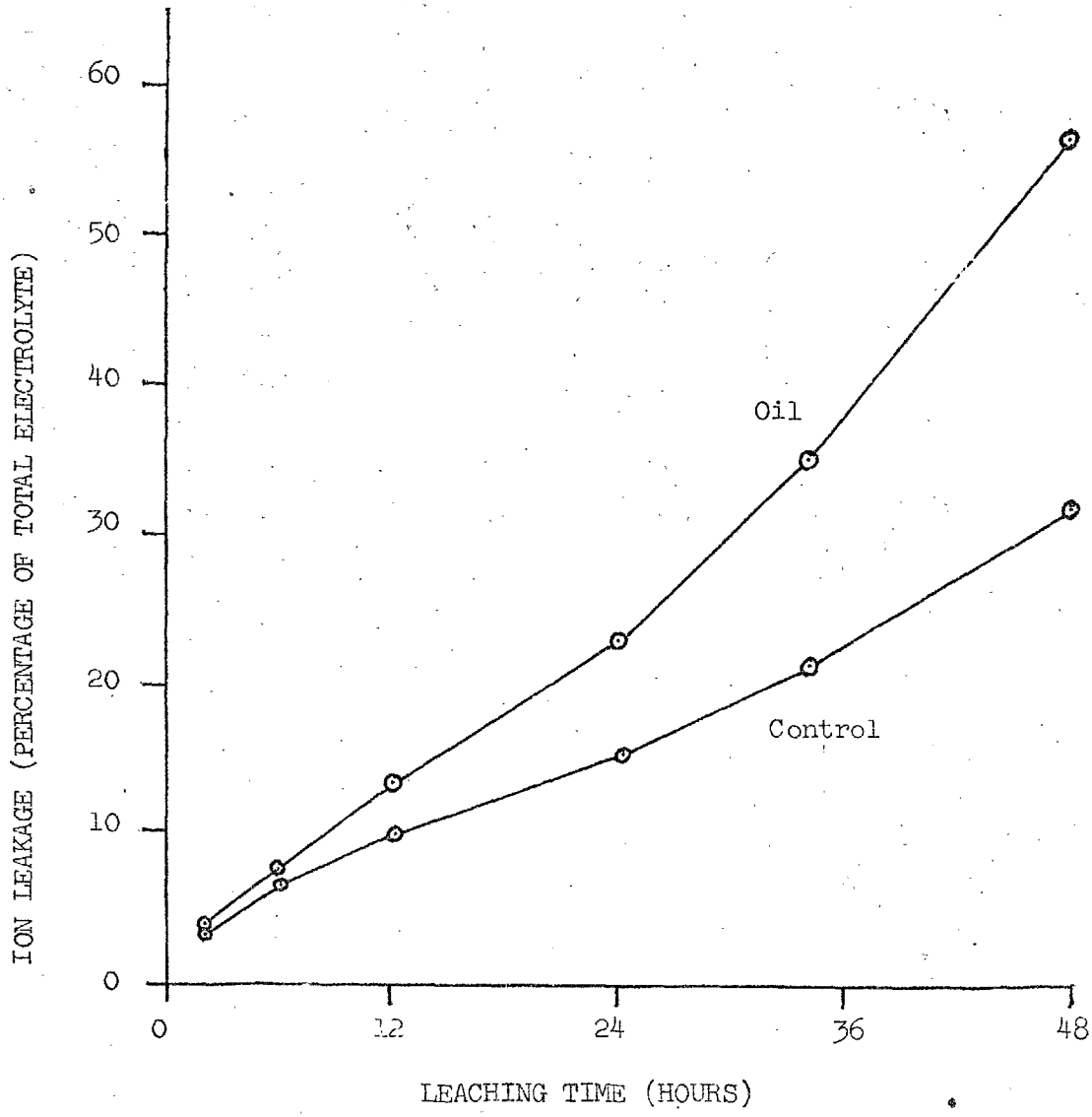
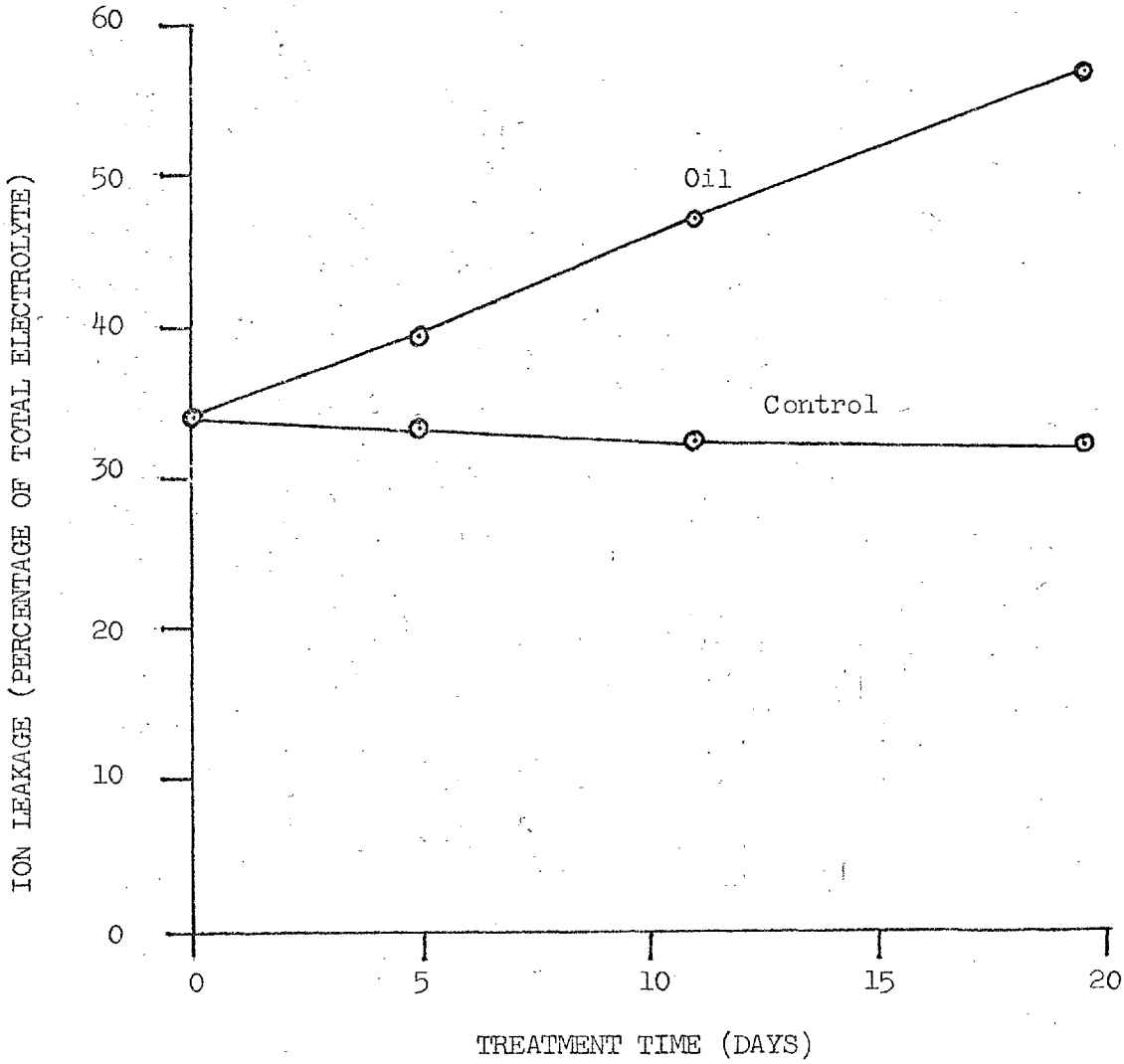


Fig. 23 The effect of treatment time on ion leakage from control and BP85 sprayed leaf samples



by Boyles (1967). He used the ion leakage technique to study the mechanism of toxicity of n-decane to carrot and sunflower leaves and found significant increases in leakage of 100 % within three hours.

F. The Effect of (N + P) Components of Experimental Oil Fractions on Mildew using Leaf Discs

A previous experiment with wide distillation range oils suggested that under certain conditions there was an inverse linear relationship between disease control and oil viscosity whereas the relationship with boiling point was curvilinear. It was shown that oils with lower boiling points than BP85 were considerably more effective in controlling the disease. Thus to investigate these points more fully a series of narrow distillation range oil fractions from which the unsaturated hydrocarbon components had been removed were tested against the disease. Fractions 13, 16, 19, 22 and 24 were prepared as described previously and their viscosities determined at 37.8°C and given in Appendix Ib. In the first experiment disease control and phytotoxicity were compared on the basis of distillation temperature. As the lower boiling fractions were highly volatile (Appendix Ic) a second experiment was carried out to determine their vapour action on the disease.

1. Disease control and phytotoxicity

Leaf discs were sprayed at levels of 0.4, 0.8 and 1.2 ml, inoculated, incubated in closed Petri-dishes and disease cover assessed as usual. Phytotoxicity, measured in terms of the total effect of oil treatment and infection, was estimated on a scale from 0 to 6 as described previously. The relationship between oil toxicity as expressed as percentage disease cover and phytotoxicity is given in Fig. 24, and between disease control and distillation temperature in Fig. 25.

Discussion

Fractions 13 and 16 were by far the most effective against the disease but were also highly phytotoxic. Fractions 22 and 24, although causing no visible signs of phytotoxicity were comparatively ineffective against the disease.

Fig. 24. The effect of (N + P) components of oil fractions on disease cover and phytotoxicity

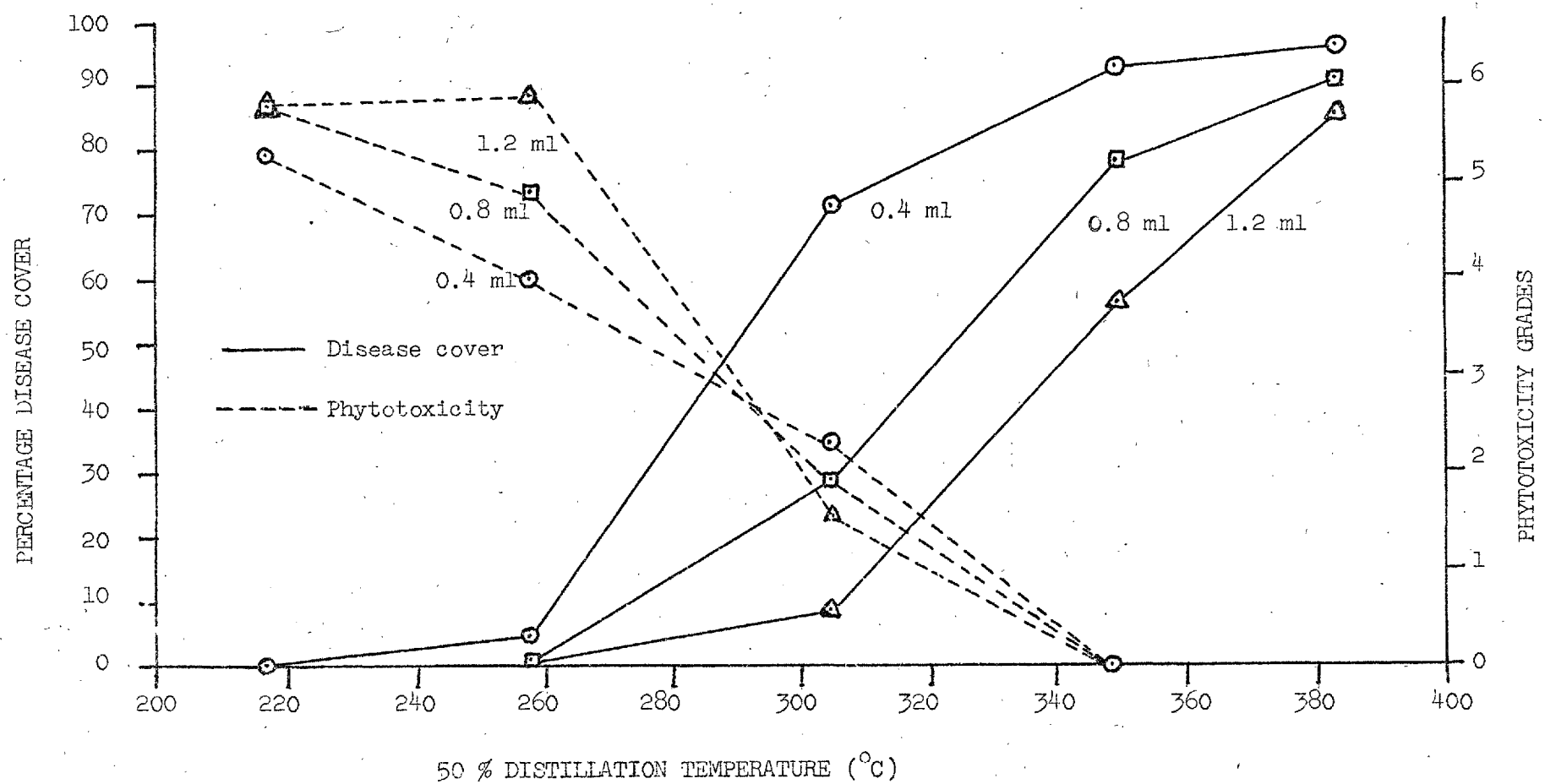
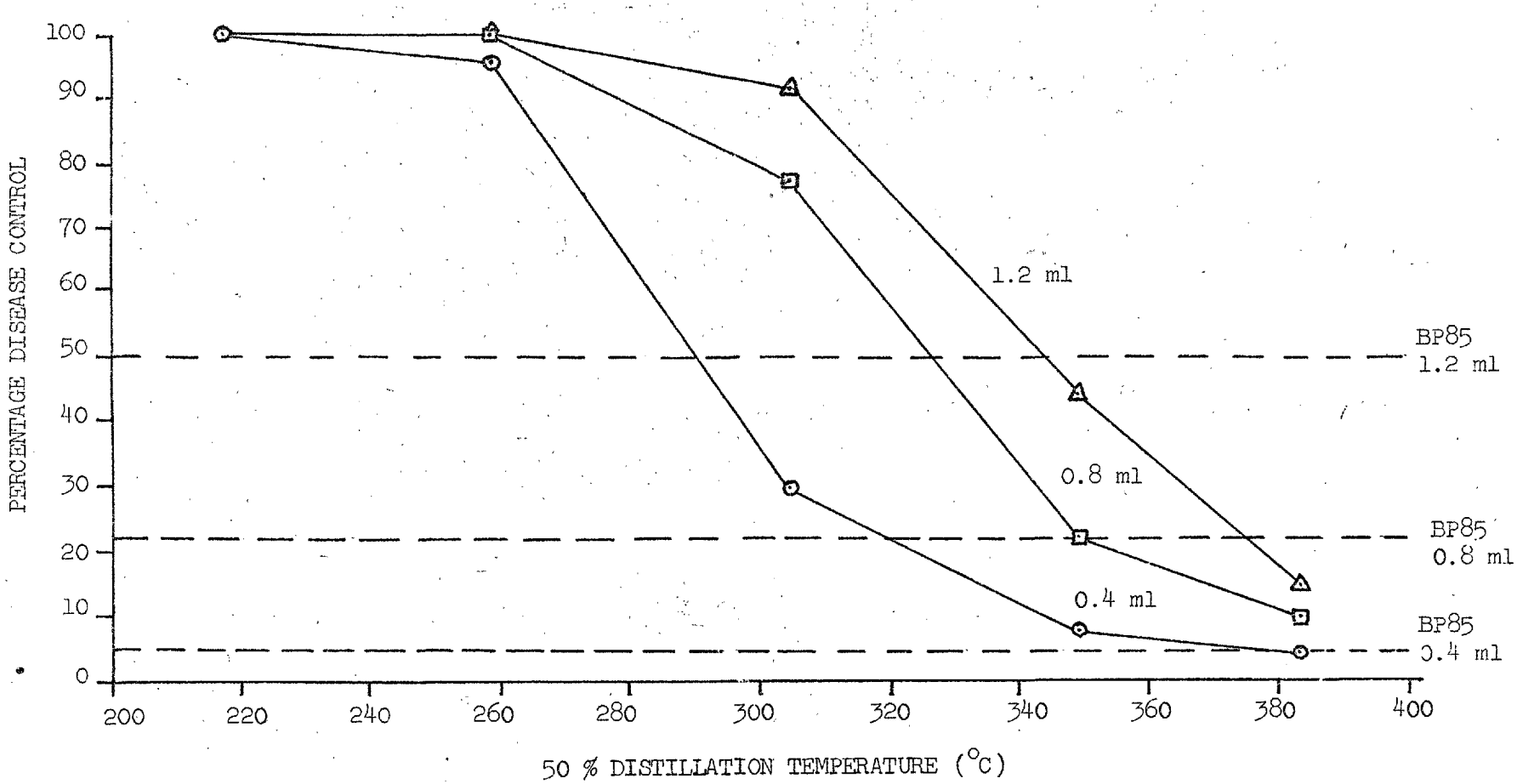


FIG. 25. The relationship between distillation temperature and disease control



Fraction 19 was intermediate with respect to disease control and phytotoxicity. At the 0.4 ml level disease control was poor and thus total phytotoxicity fairly high whereas at the 1.2 ml level disease control was more than 90 % with only slight signs of phytotoxicity.

Fig. 25 shows an almost linear relationship between 50% distillation temperature and disease control at the 1.2 ml level for fractions 19, 22 and 24. The more curvilinear relationship at the 0.8 and 0.4 ml levels may be partly due to the differences in deposit with different oils. Thus, as shown in Appendix Id, at the 0.8 ml levels, fractions 22, 19 and 13 give deposits of respectively, 24.8, 28.6 and 33.4 mg/100 cm^2. Whereas, in contrast to the lower boiling point, wide distillation range oils which fell off in toxicity at the 0.8 and 0.4 ml levels, the low boiling point fraction 19 increased in toxicity at a greater rate than the higher boiling fractions. This difference may be explained on the basis of the comparative volatilities of Fraction 19 and gas oil. Thus fraction 19 is considerably less volatile than gas oil (Appendix Ic) and thus would persist sufficiently for it to be able to exert more of its potential toxic action on the fungus than would gas oil.

The increasingly curvilinear pattern of disease control at the 1.2, 0.8 and 0.4 ml levels is clearly illustrated by the ratio of the change in percentage control between fractions 24 and 22 and between fractions 22 and 19, at these levels. The ratios are respectively, 1 to 1.6, 1 to 4.4, and 1 to 6.4. The ratios for changes in inverse viscosity and 50 % boiling point are the same i.e. 1 to 1.3. Thus for narrow distillation range oil fractions boiling point and inverse viscosity are equally valid measures of the comparative toxicities of the fractions within the very limited distillation range encompassed by the three oils.

b) <u>The vapour action of oil fractions</u>

Vapour action was determined by placing leaf discs sprayed and inoculated in the usual way on one half of a Petri-dish containing water agar. Inoculated but unsprayed discs were placed on the other half and the dish

closed to prevent vapour escaping too quickly. The results for disease control on both sprayed and unsprayed discs are given in Fig.26.

Discussion

The vapour action of fractions 13 and 16 was quite marked with fraction 19 showing only a slight effect. This suggests that in the immediate area of the oil drop and fungus there would be two modes of action of fraction 19. First a very marked effect of the oil per se. on the fungus and secondly a smaller effect due to the vapour from the oil. This latter would be expected to be greater than detected in this experiment where the test fungus was at a relatively long distance from the source of the vapour. No vapour action was detected for fraction 22 although one would expect some slight effect due to the slight volatility of the oil (Appendix Ic).

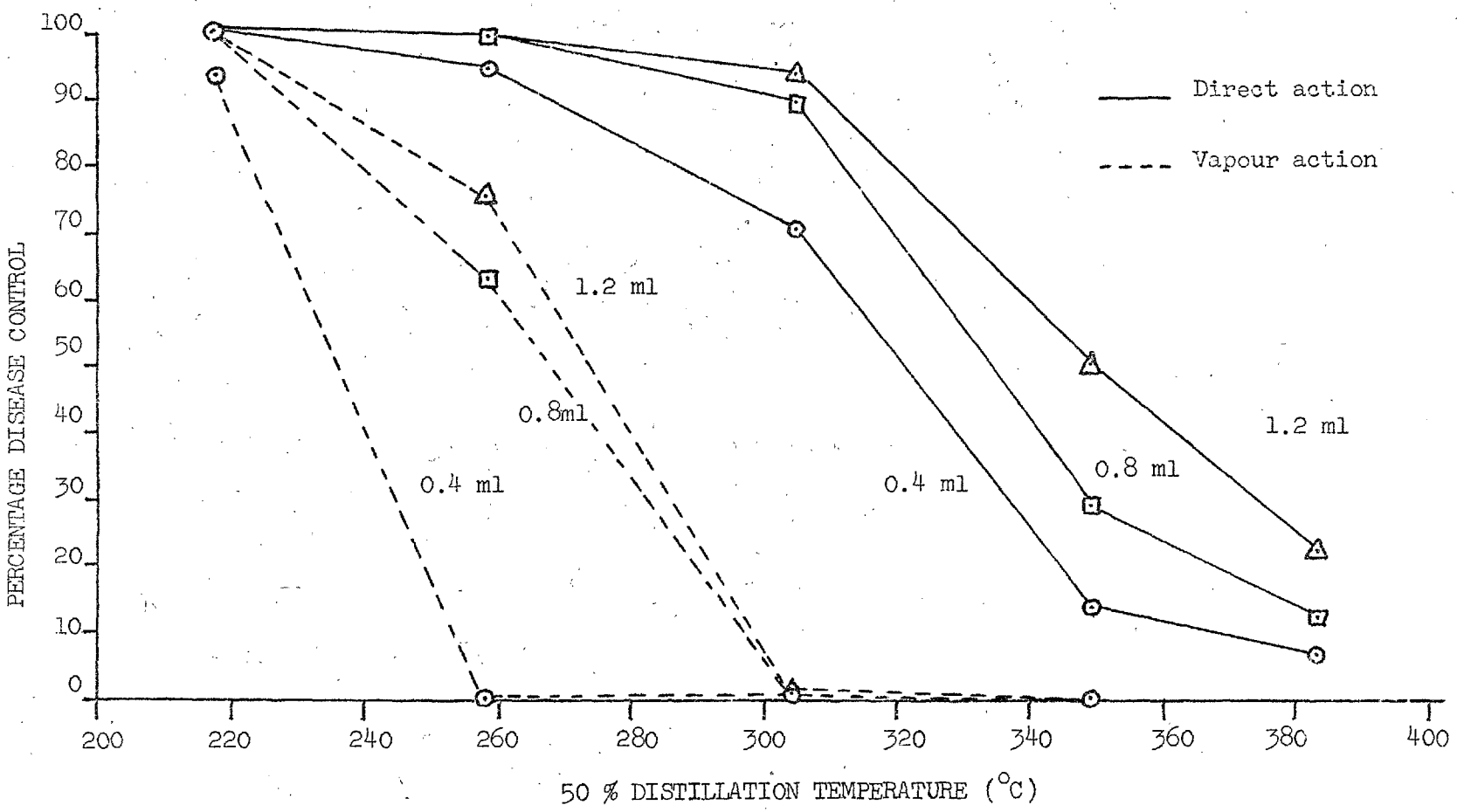
The results of both experiments described above show that the action of oil against mildew is not related to just one physical characteristic since the relationship between distillation temperature, viscosity and volatility all of which have been shown to be related to the activity of oil, are not linear.

G. The Phytotoxicity of (N + P) Components of Experimental Oil Fractions on Leaf Discs

Earlier experiments with BP85 suggested that leaf disc senescence, as measured by the chlorophyll content of leaf discs, was a fairly sensitive test of oil phytotoxicity. This method was therefore used as a test for the (N + P) components of oil fractions. As oil soaking was another symptom of oil phytotoxicity this was also measured in terms of the percentage leaf area affected using the Horsfall-Barratt grading system as described before.

Leaf discs were cut and sprayed with fractions 13, 16, 19, 22 and 24 at the 0.4, 0.8 and 1.2 ml levels as usual. The chlorophyll content of control discs was measured at the start of the experiment and at the end when the oil treated discs were measured and also estimated for oil soaking. All discs

Fig. 26. The vapour action of (N + P) components of oil fractions on cucumber powdery mildew control



were maintained on water agar and were cut in pairs from each of ten leaves and arranged so that discs from the same leaves could be easily identified. The results for both chlorophyll content and oil soaking are given in Fig.27 with BP85 included at its 50 % distillation temperature and indicated by the vertical line at this point. The three horizontal lines crossing this line and marked 4, 8 and 12 indicate the chlorophyll content at the levels of 0.4, 0.8 and 1.2. ml .

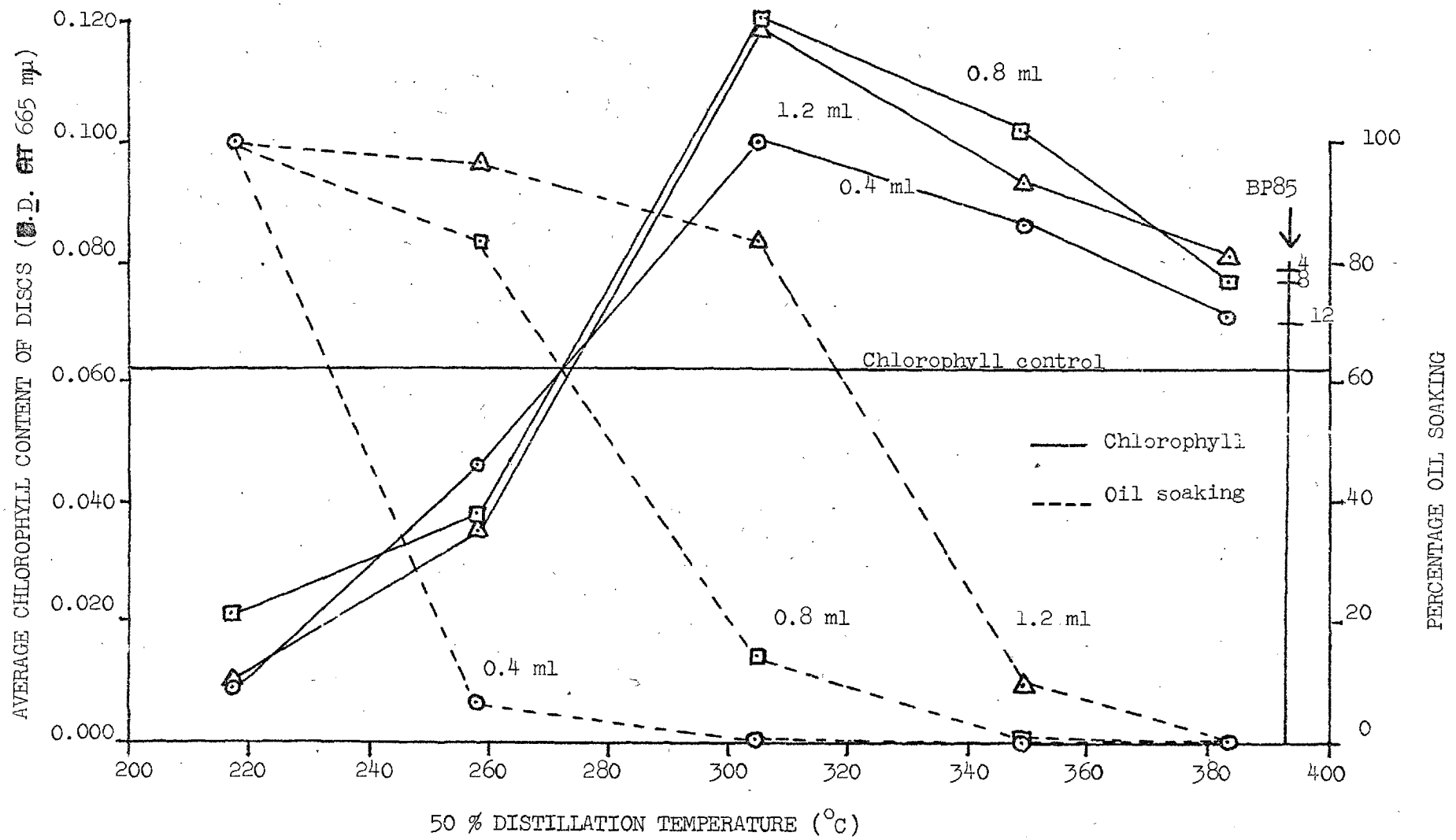
Discussion

Fractions 13 and 16 signigicantly reduced the chlorophyll content below the controls whilst fractions 19 and 22 significantly increased the chlorophyll content of discs over the controls. No significant differences were found for fraction 24 or BP85. Oil level had no significant effect on chlorophyll content except with fraction 19 where oil levels of 0.8 and 1.2 ml gave a significantly higher value than the 0.4 ml level. Differences in oil soaking, however, were equally caused by differences in oil level and distillation temperature. The results suggest that fraction 19 is considerably phytotoxic except at levels of 0.4 ml or below. The results also cast some doubt on the validity of the chlorophyll assay method as a measure of phytotoxicity. Thus while fraction 19 caused an increase over that of the control particularly at the 0.8 and 1.2 ml levels, the same levels caused 14.2 and 83.2 % oil soaking respectively. The significance of the effect of fractions 19 and 22 on chlorophyll content is thus not clear particularly as the effect is relatively small when compared with the control value of 0.455 at the beginning of the experiment. The results do, however, again confirm the results of Saunders and Lonnecker (1967) that oils do reduce the senescence of plant material. They also indicate that oils lighter than BP85 will cause a much greater effect than this oil.

H. The Effect of Unsaturated (A + H) Hydrocarbon Components of Oil Fractions on Mildew using Leaf Discs

Previous experiments showed the saturated (N + P) hydrocarbon components of oil fractions to be relatively ineffective in controlling the disease

Fig. 27. The phytotoxicity of (N + P) components of oil fractions



except at high levels where phytotoxicity was also high. It was thought possible, therefore, that the unsaturated aromatic hydrocarbon components of oil fractions, which show the highest phytotoxicity of all hydrocarbons (Havis, 1950), might be sufficiently active against the disease to be used at very low levels at which phytotoxicity might be avoided. The (A + H) components of fractions 13, 16, 19, 22 and 24 were thus tested for both disease control and phytotoxicity.

1. The minimum oil level for activity

To determine the lowest application rate at which the oils could be used to give good disease control, the five fractions were applied to leaf discs, using the pipette technique with the Potter tower described previously, at levels of 0.03, 0.05, 0.10, 0.20 and 0.30 ml . For fractions 19, 22 and 24 these levels gave oil deposits of c. 0.5, 1.2, 3.8, 9.6 and 16 mg/100 cm^2 precise values for each oil being given in Appendix Id. Values were not obtained for fractions 13 and 16 as they were very volatile. Discs were inoculated and incubated as before and the results expressed as percentage disease control in Table 70.

Table 70 The effect of (A + H) oil fraction components on cucumber powdery mildew (% disease control)

Oil level	Fraction number				
(ml)	13	16	19	22	24
0.03	0	0	0	< 1	1.3
0.05	0	0	< 1	< 1	3.7
0.10	*	*	2.1	19.0	20.4
0.20	*	*	25.0	63.0	60.0
0.30	*	*	90.0	91.8	93.4

* not estimated due to heavy phytotoxicity

Discussion

Neither of the two lowest levels caused any significant control except for the 0.05 ml level of fraction 24. Good disease control of over 90 % was only obtained with the 0.3 ml levels of fractions 19, 22 and 24 whilst the phytotoxicity caused by fractions 13 and 16 at levels of 0.10 ml and above was so high as to prevent disease estimations. Thus fractions 19, 22 and 24 seemed the most promising and were therefore tested further.

2. Disease control and phytotoxicity with (A + H) components of fractions 19, 22 and 24

Fractions 19, 22 and 24 were sprayed at levels of 0.1, 0.2, 0.3 and 0.5 ml to confirm their action on the disease and their phytotoxicity both in terms of oil soaking and chlorophyll content. Oil soaking was estimated after 1 and 9 d and chlorophyll at the end of the experiment at 9 d. Results are given in Table 71.

Table 71 Disease control and phytotoxicity with (A + H) components of fractions 19, 22 and 24

Treatment		%	Phytotoxicity			
Fraction number	Oil level (ml)	Disease control	Oil soaking (%) Day 1	Oil soaking (%) Day 9	Chlorophyll at 9 d D at 665mμ	Chlorophyll at 9 d % Reduction
19	0.1	—	1.9	56.0	0.046	60.3
	0.2	100*(1)	5.0	100	* *	100
	0.3	* *	6.2	100	* *	100
	0.5	* *	18.0	100	* *	100
22	0.1	87.0	0	8.8	0.084	27.6
	0.2	100*(2)	<1	43.0	0.036	69.0
	0.3	100*(3)	<1	98.5	0.004	96.6
	0.5	* *	<1	100	* *	100

Table 71 cont.

	0.1	84.0	0	1.5	0.110	5.2
	0.2	96.7	0	3.6	0.102	12.1
24	0.3	>99	<1	7.0	0.079	31.9
	0.5	100	<1	11.6	0.074	36.2
Disease	control	0	—	—	—	—
Healthy	control	—	0	0	0 116	0

* ( ) average of number of replicates in brackets, due to phytotoxicity

* * not estimated due to phytotoxicity

Discussion

In this experiment fraction 19 caused severe phytotoxicity even at the lowest level of 0.1 ml and was not used in any further tests. Fraction 24 caused no significant phytotoxicity in terms of chlorophyll reduction at the 0.1 and 0.2 ml levels and only slight oil soaking. Disease control was also very good but the oil itself was very viscous (Appendix Ib) and thus difficult to spray. Of the fractions available at this time, fraction 22 showed the best compromise between disease control, phytotoxicity and ease of spraying.

The effect of distillation temperature on the type of phytotoxicity caused by oils was clearly shown by the three fractions. Thus fraction 19 caused a very rapid chlorosis so that by 3 d most of the discs at levels of 0.2 to 0.5 ml were completely colourless. Fraction 22 caused a similar type of phytotoxicity but which progressed at a much slower rate so that at 3 d most of the discs were still green though markedly less than the controls. Phytotoxic symptoms for fraction 24, were confined to small necrotic spots so that whereas by 9 d discs sprayed at the 0.1 ml level with this fraction showed similar necrotic spotting to the same level of fraction 22, the chlorophyll content of discs sprayed with the former oil was not significantly different from the control discs.

Fraction 22, however, caused considerable loss of chlorophyll. Unlike fraction 22, fraction 24 did not cause any large areas to become completely chlorotic even at the high levels of 0.3 and 0.5 ml .

3. Disease control and phytotoxicity with (A + H) components of fractions 21, 22 and 23

At a much later date to the previous experiment the (A + H) components of fractions on either side of fractions 22 became available and were therefore compared at the 0.15 ml rate for disease control and phytotoxicity. In the first experiment they were sprayed at the time of inoculation and in a second smaller experiment just before sporulation. Disease cover and chlorophyll content of infected discs were measured as before and given in Tables 72 and 73.

Table 72 Disease cover and chlorophyll content of discs sprayed with (A + H) components of fractions 21,22 and 23 at the time of inoculation

Fraction number	% Disease cover	% Control	Chlorophyll (D at 665mμ)
Control	100	0	0.044
21	74.8	25.2	0.006
22	35.0	65.0	0.015
23	46.0	54.0	0.035

Table 73 Chlorophyll content of discs sprayed with (A + H) components of fractions 21, 22 and 23 just prior to sporulation

Fraction number	Chlorophyll (D at 665mμ)
21	0.004
22	0.015
23	0.069

Discussion

Although disease was reduced significantly, the oil fractions 21 and 23 significantly reduced the chlorophyll content of discs below that of the diseased controls. Fraction 23 did not, however, cause any significant difference but uninfected discs were much yellower (i.e. senescent) than the healthy controls indicating phytotoxicity. Fractions 22 and 23 did not differ significantly indisease control but both were significantly more effective than fraction 21. The much lower phytotoxicity of fraction 23 was confirmed by spraying infected discs just prior to sporulation. Examination under a stereo-zoom microscope showed that fractions 21 and 22 completely prevented sporulation whereas only 60 % of discs sprayed with fraction 23 were completely prevented from sporulating. Due to the relatively poor survival of cucumber leaf discs it was not possible to decide whether fraction 23 per se. was less toxic to the fungus or whether the greater phytotoxicity of fraction 22 prevented the leaf discs treated with this oil from supporting sporulation.

3. A comparison of hydrocarbon components of fraction 22

As fraction 23 was not available at the time of this work, fraction 22 was the most promising oil available. The hydrocarbon components of this fraction, as described in Materials and Methods, Section D.2 b) and Fig. 5, were therefore compared for both disease control and total damage caused by the oil treatment plus disease (T. + D.). The latter was assessed using the Horsfall-Barratt grading system to measure oil or water soaked areas. The components were the aromatics plus heterocyclics (A + H), naphthenes plus paraffins (N + P), crude oil (C.O.) consisting of the latter two components plus the total wax content of the fraction, dewaxed oil process I (D.W.O. I) consisting of the (N + P) component minus its wax content and dewaxed oil process II (D.W.O.II) consisting of crude oil minus its wax content. They were sprayed at the 0.1, 0.2, 0.3 and 0.5 ml levels, discs inoculated and incubated as before. The results are given in Table 74.

Table 74 A comparison of fraction 22 component treatment

Percentage disease control

Oil level (ml)	Hydrocarbon component A + H	N + P	D.W.O. I	C.O.	D.W.O. II
0.1	29.0	3.4	2.4	4.8	5.4
0.2	91.9	3.4	4.5	31.0	38.0
0.3	> 99	5.2	5.2	60.0	93.4
0.5	100	5.6	6.8	99.0	100

Percentage total damage (T. + D.)

Oil level (ml)	Hydrocarbon component A + H	N + P	D.W.O. I	C.O.	D.W.O. II
0.1	< 1	87.2	* *	* *	43.0
0.2	3.9	91.6	* *	6.6	10.8
0.3	8.8	89.0	* *	7.6	80.8
0.5	91.6	85.6	* *	97.6	86.6

* * not estimated

Discussion

As expected, the (A + H) component as by far the most effective treatment. It was interesting also that the crude oil component put through the wax extration process (i.e. D.W.O. II) was more fungitoxic and phytotoxic than before the process (i.e. C.O.). It was noticed that the dewaxed (N + P) fraction was slightly coloured and also that the dewaxed crude oil was darker in colour than the ordinary crude oil. Thus the dewaxing process would seem to have

increased the toxicity of the oil. As the dewaxed (N + P) component was not, however, any more active against the disease than the (N + P) component per se., it would seem that the dewaxing process only had a significant biological effect on the unsaturated hydrocarbons. The results emphasize the need for care in treating oils for biological uses.

4. The effect of oil dilution on disease control and phytotoxicity

Although phytotoxicity caused by fraction 22 was low compared with lower boiling point fractions it was still too high for commercial use. Thus it would be desirable to dilute the fraction with a non-phytotoxic carrier oil which would reduce phytotoxicity but maintain good disease control.

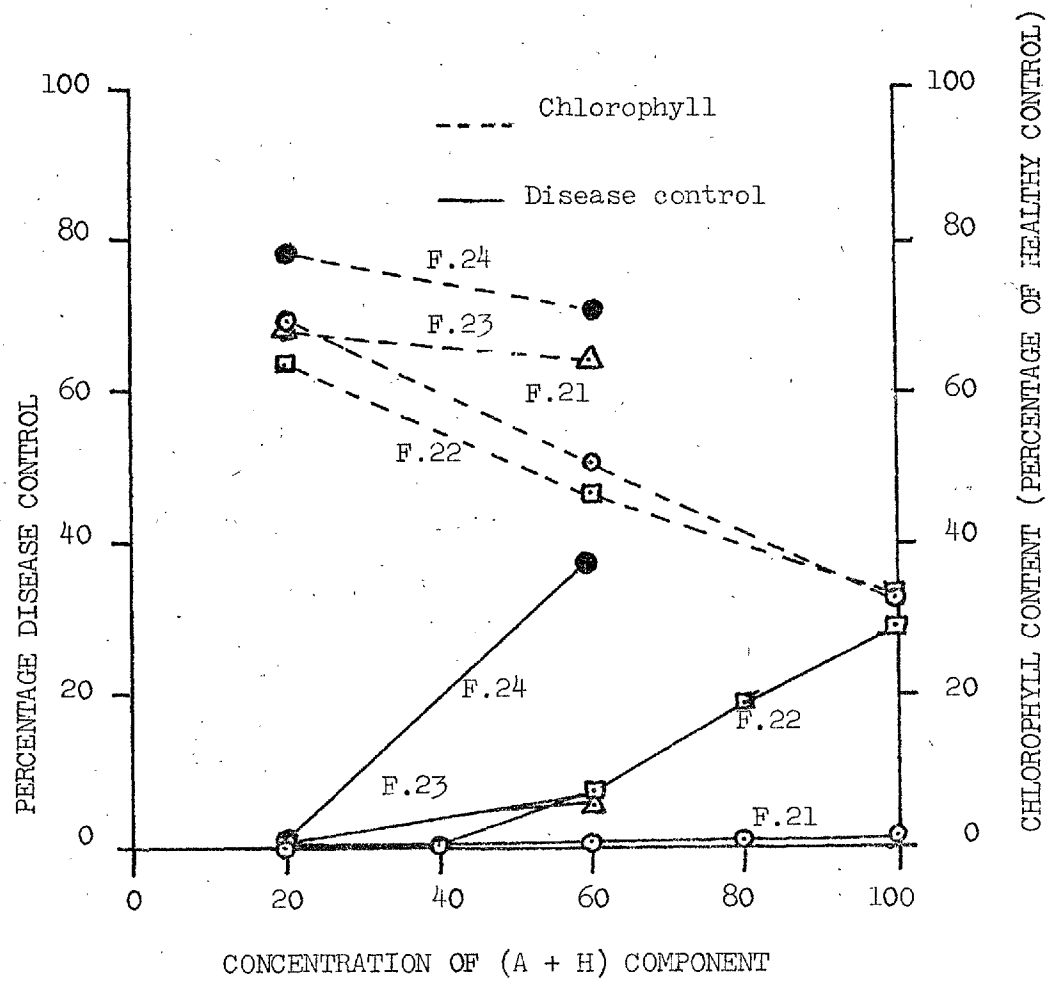
Fractions 21, 22, 23 and 24 were diluted in BP85 giving concentrations of from 20 % to 100 % and sprayed on leaf discs at the 0.15 ml level. For disease control fractions 21 and 22 were tested at the 20, 40, 60, 80 and 100 % concentrations and fractions 23 and 24 at the 20 and 60 % dilutions. For phytotoxicity all four fractions were tested at the 20, 60 and 100 % concentrations and determined on uninfected discs in terms of oil soaking and chlorophyll content as before. Results are given in Table 75, and Fig. 28.

Table 75 The effect of oil fraction dilution on oil soaking (percentage leaf area affected)

Fraction	% (A + H)		
number	20	60	100
21	< 1	< 1	1.0
22	< 1	1.7	1.8
23	< 1	< 1	*
24	< 1	1.0	*

* not tested

Fig. 28 The effect of oil dilution on disease control and chlorophyll content



Discussion

Of the oils tested fractions 22 and 21 were the most phytotoxic with fraction 21 giving virtually no disease control. At a concentration of 60 % fraction 24 gave by far the greatest disease control and was also the least phytotoxic causing only a 30 % reduction whilst fraction 22 caused a 53.4 % reduction. Although fraction 24 at 60 % did not differ significantly in disease control from fraction 22 at 100 % the results of a previous experiment, where the two fractions did not differ significantly at 100 %. suggest that dilution, at least down to 60 %, does not reduce the activity of this fraction but does make spraying far easier. Thus, fraction 24 at 100 % had a viscosity of c. 470 centistokes whilst at 60 % its viscosity was only c. 100 centistokes (Appendix I b). It is clear, however, that dilution to values at which disease control might still be acceptable commercially does not completely reduce phytotoxicity on leaf discs.

Values for disease control in this experiment were very low and since in previous experiments the same treatments had given much higher values it was thought likely that variations in inoculum level might be the reason. This was tested in the following experiment.

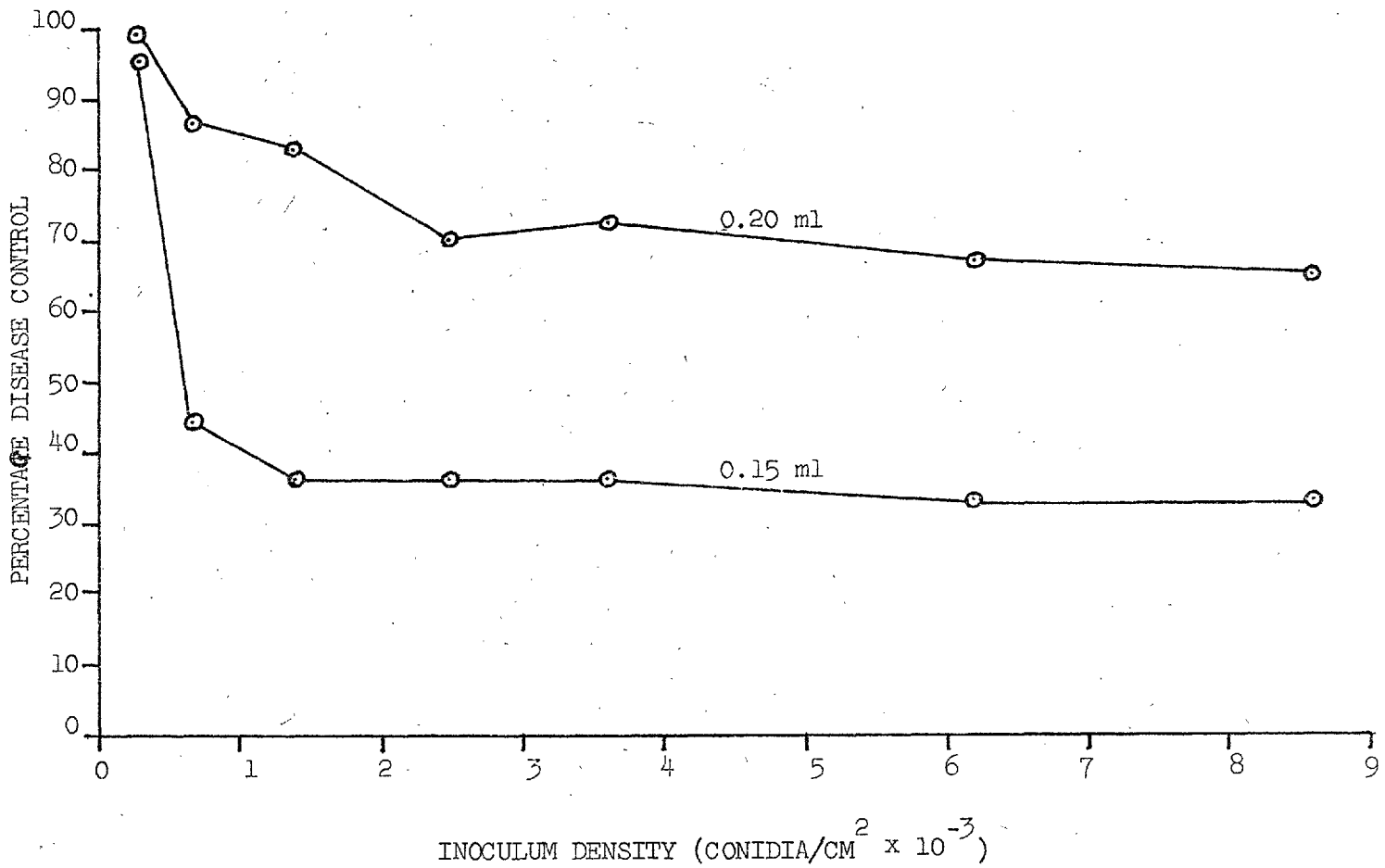
5. The effect of variations in conidial inoculum density on disease control

Variations in inoculum density were produced as before using whole plants as an inoculum source. Fraction 22 was sprayed at the 0.15 and 0.20 ml levels and disease cover estimated as usual. Results are given in Fig. 29.

Discussion

As found with BP85, disease control felloff with increasing inoculum level. The pattern of the effect was also very similar to that found for BP85. Thus at low inoculum levels both oil levels gave high disease control whereas with higher inoculum levels the difference in disease control between the two oil levels increased to between 30 and 35%, thereafter, remaining constant. The threshold range for the effect of decreasing inoculum level on disease control was, as for BP85, between 500 and 1,000 conidia/cm^2 with the effect being much smaller at the higher oil level.

Fig. 29. The effect of conidial inoculum density on disease control



The main difference between the (A + H) component of fraction 22 and BP85 was the much higher activity of the former. Thus at an inoculum level of 250 conidia/cm^2 fraction 22 gave c. 95 % disease control at an oil level of 0.15 ml (c. 6 mg/100 cm^2) whereas BP85 gave only c. 80 % control at an oil level of 1.2 ml (63 mg/100 cm^2). Fraction 22 also maintained its activity after the initial fall off indicating that it was highly toxic to the fungal mycelium whereas further experiments showed that at the 0.2 ml level the oil had no significant effect on germination. Thus the oil protected a much greater area than it actually covered. The threshold effect could therefore be explained by the ability of the oil not only to inhibit the growth of fungal hyphae but also to inhibit or slow down the growth of new germ tubes and hyphae from the original conidium. At low inoculum levels this would leave only a small number of germinating conidia initially unaffected by the oil and which would be incapable of completely colonizing the unprotected leaf surface. At higher inoculum levels the number of germinating conidia initially unaffected by the oil would be much higher thus being able to fully colonize the unprotected leaf area and giving a constant value for disease cover from the threshold inoculum range upwards.

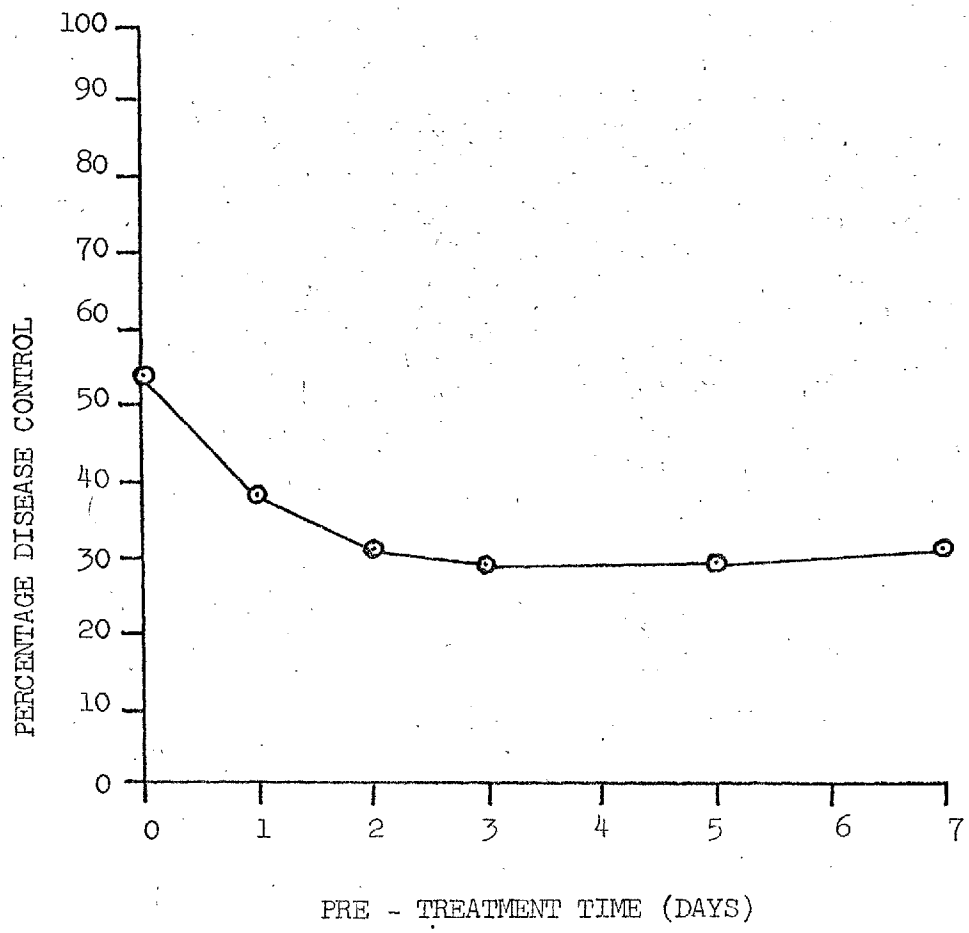
6. The persistence of the (A + H) component of fraction 22

Although fraction 22 was of low volatility and was therefore unlikely to show any loss of activity due to volatilization, it was possible that other factors, such as the loss of oil from the leaf surface into the leaf tissue, might affect its activity. To test this the oil was sprayed at the 0.1 ml level at 0, 1, 2, 3, 5 and 7 d prior to inoculation. Disease cover was estimated as before, and results, expressed as percentage disease control, given in Fig. 30.

Discussion

Disease control fell off soon after spraying and by 2 d had been significantly reduced by 42.6 %. Clearly this would be an unacceptable characteristic for the use of the oil as a fungicide.

Fig. 30 The effect of pre-inoculations sprays of fraction 22, (A + H) component, on disease control



7. The eradicant action of the (A + H) component of fraction 22

At the low spray rates used for (A + H) components, the amount of leaf area covered with the oil would be very small and thus its effect on conidial germination similarly small. As disease control was, however, high the effect of the oil on the mycelium must have been similarly high. This, together with the results of the last experiment where activity fell off with time on the plant, suggests that disease control might be improved by spraying after inoculation. Thus the oil in its most active state would immediately come into contact with germ tubes and hyphae of the fungus. To test this, fraction 22 was sprayed at the 0.2 ml level immediately after inoculation and 12, 26, 36, 48, 72 and 96 h later. The results expressed as percentage disease control are given in Fig. 31.

Discussion

Disease control was significantly improved by 15.8 and 18.4 % by spraying at 26 and 48 h after inoculation. With the 72 h treatment control had decreased slightly and with the 96 h spray was not significantly different from that produced by the 0 h spray. In the ~~latter~~ 96h case the mycelium had developed sufficiently before treatment to be included in the final estimate of mycelial coverage.

I. The Effect of the (A + H) Component of Fraction 22 on Sphaerotheca fuliginea In Vivo

The celloidin peel technique was used to determine the effect of the oil applied at the 0.2 ml level on both the conidia and mycelium of the fungus. Discs were sprayed, inoculated and incubated as before. Peels were taken at 24 and 48 h and disease cover assessed at 8 d as usual. Results for the effect on the length of 1^{o}, 2^{o} and 3^{o} hyphae at 48 h are given in Table 76 and for the effect on hyphal production expressed as a percentage of germinated conidia given in Fig. 32. Hyphal length was measured using conidia with at least three germ tubes.

Fig. 31 The effect of post-inoculations sprays of fraction 22, (A + H) component, on disease control

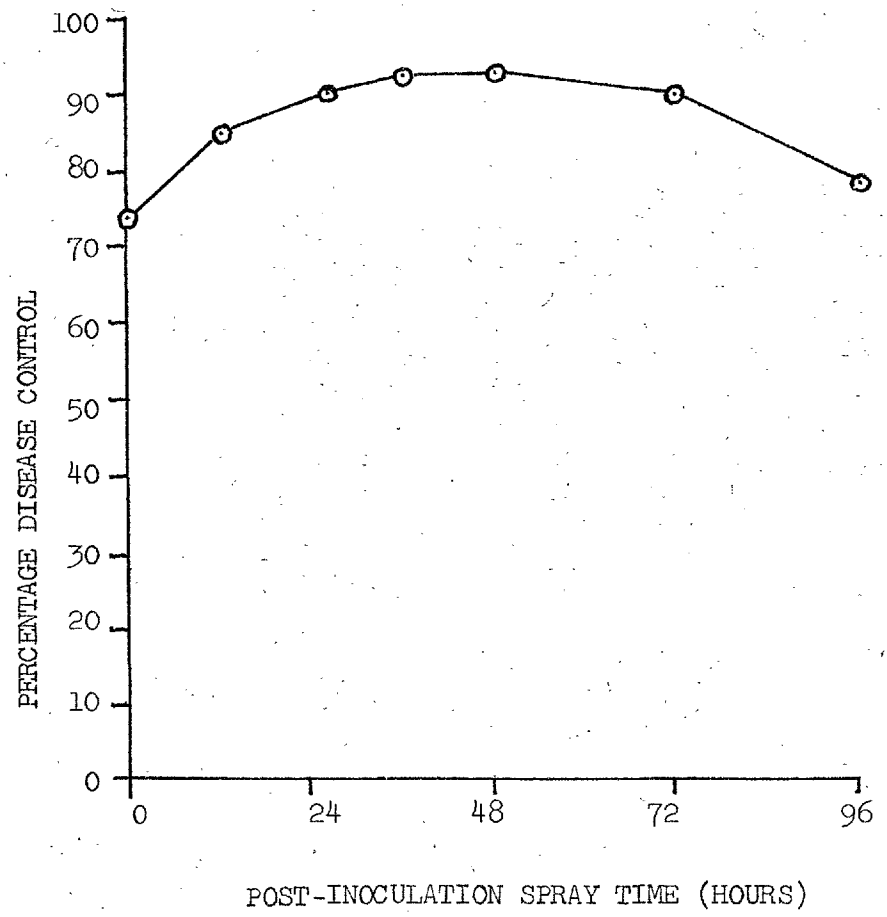


Table 76 The effect of fraction 22 (A + H) component on hyphal growth

Hyphal type	Hyphal length (eye piece units)		% growth
	Control	Oil	reduction
1^{o}h	6.9	3.3	52.2
2^{o}h	3.9	1.6	59.0
3^{o}h	2.0	1.1	45.0

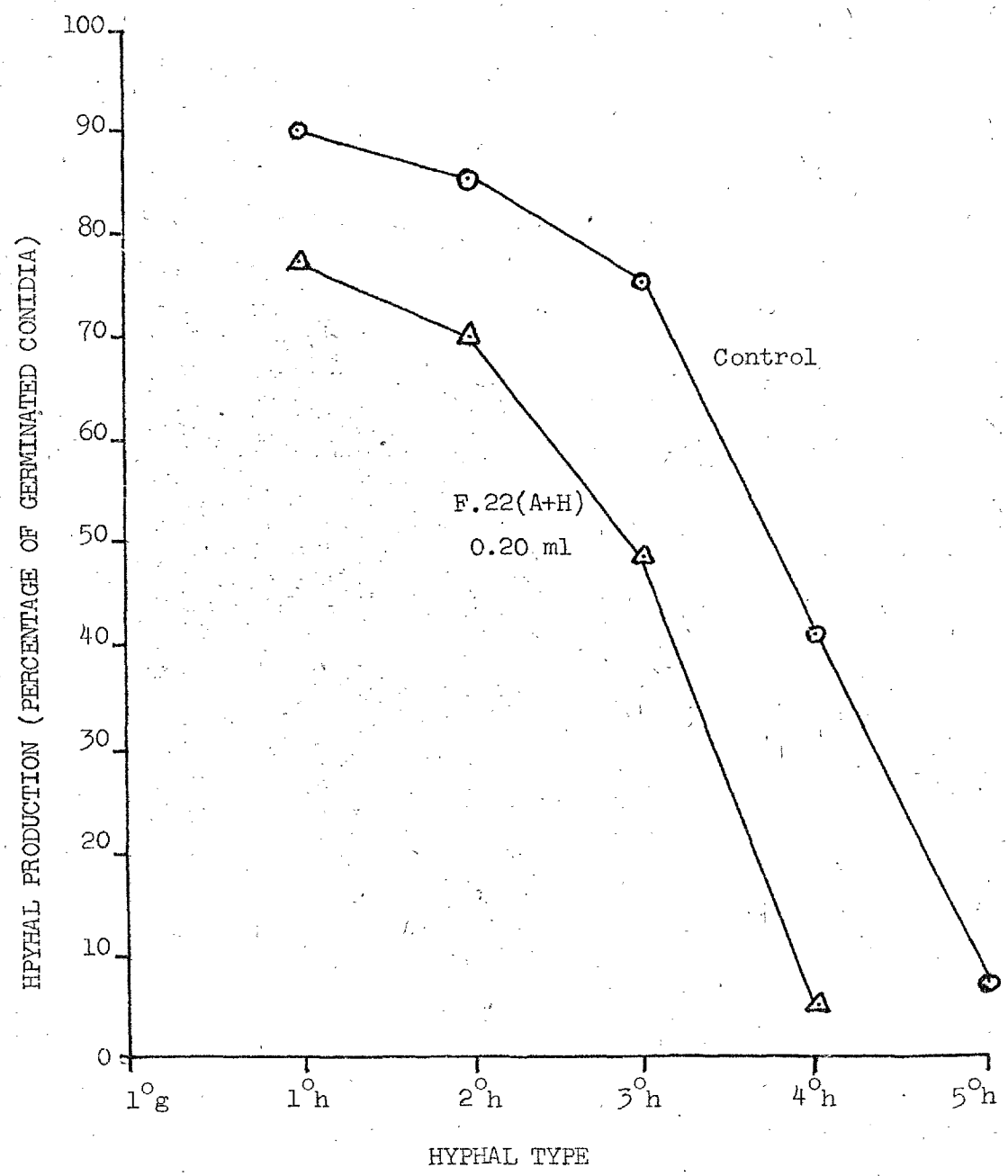
Discussion

Fraction 22 at 0.2 ml had no signigicant effect on germination whereas by 48 h the growth of hyphae was reduced by c. 50 % which correlated well with disease control which was 54.0 %. Hyphal production at 48 h was reduced by a comparatively small amount being 13.7, 17.9 and 34.2 % for 1^{o}, 2^{o} and 3^{o} hyphae. As can be seen from these figures and Fig. 32 the effect on hyphal production gradually built up with the production of 4^{o} hyphae being reduced by 88.1 %. It would appear therefore that the effect of the oil on the hyphae was transmitted in some way to the spore which consequently failed to produce new hyphae. The effect of the oil on the disease would therefore be a result of both reduced growth and production of hyphae.

J. The Phytotoxicity and Persistence of the (A + H) Components of Oil Fractions on Whole Plants

At the time of this experiment there was insufficient fraction 22 available to spray whole plants. Fractions 19 and 21 therefore were used with the Micron ULVA at the 1.0 ml levels giving deposits of 2.9 and 4.9 mg/100 cm^{2} respectively. The plants were kept in the greenhouse at temperatures varying between c. 15°C to c.35°C for 12 d. At this time the persistent activity of the oils was determined by inoculating plants using a

Fig. 32 The effect of fraction 22 (A + H) component on hyphal production by Sphaerotheca fuliginea



large settling tower. Treatment replicates were arranged randomly in an attempt to compensate for variations in inoculum level. Disease cover was determined by cutting 10 mm discs from inoculated leaves and assessing these in the usual way. Results are given in Tables 77 and 78.

Table 77 The Phytotoxicity of (A + H) components of oil fractions 19 and 22 on whole plants (% leaf area with necrotic spots)

Estimation	Fraction 19 (2.9 mg/100 cm^2)			Fraction 21 (4.9 mg/100 cm^2)		
Time (d)	Leaf 2	Leaf 3	Leaf 4	Leaf 2	Leaf 3	Leaf 4
1.5	1	1	4.1	1	1	1
4	1	1	5.0	1.0	2.2	2.2
8	1	1	5.0	7.0	10.0	4.7
8	F.21 - Light green haloes *			20.0	59.0	6.2

* see discussion

Table 78 The persistent activity of the (A + H) components of oil fractions against cucumber powdery mildew on whole plants (% disease control)

Fraction No.	Leaf No.	% Control
19	2	0
(2.9mg/100 cm^2)	3	0
	4	0
21	2	99
(4.9mg/100 cm^2)	3	99
	4	96.9

Discussion

In contrast to leaf disc experiments where fraction 19 gave good disease control but heavy phytotoxicity when applied immediately before inoculation, in the present experiment there was no effect on the disease and only slight phytotoxicity. Thus the high volatility of the oil together with the high temperature and open conditions caused the oil to volatilize and disperse completely within 12 d or at least to such an extent that any remaining had no significant effect on the disease. The slight toxicity on leaf 4 was probably due to the rapid penetration and toxicity of a small amount of the oil before it completely volatilized. The fact that this did not increase significantly over a period of 8 d shows that the effect occurred almost immediately. Being far less volatile than fraction 19, fraction 21 caused much greater phytotoxicity which increased with time suggesting a gradual penetration of the oil into the leaf tissue. With this fraction, the light brownnecrotic spots were surrounded by haloes of tissue of a lighter green than the rest of the leaf. These were also estimated at 8 d and as shown in Table 77, covered a much greater area of leaf than the necrotic spots alone. Disease control alone was almost 100 % indicating good persistence and also a low inoculum level since the same fraction gave very poor control at a similar oil level in a previous leaf disc experiment.

The Effect of Crude Oils

Undiluted (A + H) components caused heavy phytotoxicity both on leaf discs and whole plants. To test the effect of diluted (A + H) components on whole plants, the crude oil fractions 21 and 23 were used each containing 34.3 and c.40 % of their respective (A + H) components. They were sprayed onto half leaves with the Micron ULVA at the 1.0 and 2.0 ml levels giving deposits as shown in Table 79. As the oils did not produce clearly defined necrotic spots, phytotoxicity was assessed in terms of the chlorophyll content of treated half leaves, discs being cut from these and determinations made as usual. Results expressed as percentage reduction of chlorophyll content are given in Table 79.

Table 79 The effect of crude oil fractions on the chlorophyll content of cucumber leaves (% reduction)

Fraction No.	Oil level (mg/100 cm^2)	Leaf number 2	3
21	4.3	25.8	10.5
	9.2	40.6	20.5
23	3.1	27.7	17.9
	6.9	57.9	30.3

Discussion

Both oils clearly reduced the chlorophyll content of half leaves below that of control halves. Sprayed halves showed chlorotic areas which for fraction 21 were more diffuse than for fraction 23. At the higher levels these areas were more chlorotic and were also more pronounced on the older leaves (leaf No.2).

Thus, even at low dilutions, the (A + H) components still caused significant phytotoxicity and thus could not be used in their crude state in commercial practice.

K. The Effect of Fraction 22, (A + H) Component on Other Powdery Mildew Diseases

The effect of the oil on cucumber powdery mildew was compared with its effect on vine and blackcurrant powdery mildews caused by Uncinula necator and Sphaerotheca mors-uvae respectively, plant and fungal material for tests against the latter disease being obtained from R.G. Perera, Botany Department, Imperial College. Discs were cut and sprayed with oil at the 0.15 ml level as before. Inoculations were carried out using the same high inoculum level for each fungus of c. 6000 conidia/cm^2. At this level large variations in conidial inoculum density of cucumber powdery mildew had no significant effect

on disease control. Disease cover was assessed as usual, the results being given below.

Discussion

There was no significant difference in control based on mycelial coverage between any of the disease tested, the average value being 37.0 %.

SUMMARY AND DISCUSSION

The control of cucumber powdery mildew on leaf discs with BP85 at any particular level, was dependent upon the density of the conidial inoculum used. At low inoculum levels more than 90% disease control was obtained with oil levels down to 0.2 ml (11.0 mg/100 cm^2), results which are very similar to those of Castellani and Matta (1964) who obtained 95.4 and 99.7 % control with oil deposits of 8.0 and 16.0 mg/100 cm^2 respectively. At higher levels, however, control fell off to between 5 and 10 % and even at the high oil level of 63 mg/100 cm^2 control fell to between 15 and 20 %.

Gas oil with a much lower distillation temperature than BP85 gave greater control whereas 150 neutral with a higher distillation temperature gave less control. This inverse relationship between mildew control and distillation temperature was confirmed using the narrow distillation range, experimental oil fractions. Disease control fell off rapidly between c. 260°C and c.360°C with phytotoxicity showing a similar progression. However, an attempt to determine a mathematical relationship between distillation temperature or viscosity and disease control was impossible due to the high volatility of the lower distillation temperature oils. The vapour of these volatile oils were both highly fungitoxic and phytotoxic.

Other factors such as oil quality and leaf surface characteristics also affected disease control. Thus BP85 exposed to sunlight for a few months gave higher control than BP85 stored in the dark; the extreme hairiness of cucumber leaves caused a larger amount of oil to be deposited on them than on the smooth cotyledons and thus also giving greater disease control.

BP85 was persistent over a period of 8 d with control increasing with length of time on the leaf before inoculation. Post inoculation sprays showed the oil to have an eradicant effect, particularly on sporulation which was still reduced by c. 45% even when oil was applied 7 d after inoculation.

When sporulating cultures of the fungus were sprayed with the oil, spore release by air currents was restricted though even at the high level of 35.4 mg/100 cm^2 this was only 67 %. However, the very low level of 4.2 mg/100 cm^2 still caused a 28 % reduction which might be sufficient to delay an epidemic until environmental conditions became unfavourable for the disease.

BP85 applied to leaf discs at a rate of 63.0 mg/100 cm^2 reduced germination by c. 50 %, the production of hyphae by germinating spores by c. 17° % and the growth of hyphae by between c.15 and c. 30 %. The oil reduced germination on glass by c. 60 % showing a slight increase in effect compared with that on leaf discs and exposure of the oil to sunlight increased its effect on germination to 70 %.

It was suggested from the above results that the oil only inhibited germination when conidia were completely embedded in it. This would leave two populations of germinating conidia affected by oil in two different ways. The first would be in contact with the oil, although not completely embedded and thus the production and growth of hyphae would be slowed down. A second group would be affected via the hyphae when these came into contact with the oil and would thus be slowed in growth and possibly as a consequence, new hyphae would be produced more rapidly.

At the very high rate of more than 90 mg/100 cm^2, BP85 reduced plant growth although at the normal level of 8 mg/100 cm^2 no effect was detectable. The oil was also found to cause membrane damage detected by increases in the electrical conductivity of water containing oil sprayed leaf tissue. This,

however, was only studied at the high oil rate of 77 mg/100 cm^2 and suggests that it would not be significant at normal low application rates.

The unsaturated, (A + H), hydrocarbon component of oil fractions was far more fungitoxic than the saturated (N + P) component. Thus, when sprayed at a rate of c.7 mg/100 cm^2 the (A + H) component of fraction 22 gave 91.9% control whereas the (N + P) component only gave 3.4% control. The phytotoxicity of these (A + H) components was also very high, causing oil soaking, necrosis, and a reduction in leaf disc chlorophyll content. Phytotoxicity decreased with increasing distillation temperature although it was not completely eliminated. Even at a dilution of 20 % in BP85, the least phytotoxic oil tested (fraction 24) still caused a 20 % reduction in chlorophyll content. When sprayed onto whole plants at a rate of between 3.1 and 6.9 mg/100 cm^2, fraction 23 diluted to a concentration of c.40 % still caused between 17.9 and 57.9 % chlorophyll reduction. Thus the (A + H) components in their crude state could not be used commercially.

Disease control with fraction 22 was dependent upon the density of the conidial inoculum used. The pattern of the effect was very similar to that found for BP85, though at a much higher level of activity, the threshold range for the effect of decreasing inoculum level on disease control being between 500 and 1,000 conidia/cm^2. The oil was persistentfor a period of 7 d though control fell off by c. 40 % when the oil was left on the leaf surface for 2 d before inoculation. This was probably the reason for the c. 26% increase in control obtained by applying the oil between 24 and 48 h after inoculation, the relatively small deposit of oil used immediately coming into contact with the fungal mycelium when in its most active state.

When applied at a rate of 8.6 mg/100 cm^2 fraction 22 had no significant effect on germination on leaf discs but reduced the growth of hyphae at 48 h by between 45.0 and 59.0 %, which correlated well with disease control of

54.0 %. The effect on hyphal production was initially much less, being a reduction of only 13.7 % for primary hyphae. This, however, increased with consecutive hyphae, tertiary hyphal production being reduced by 34.7 % and 4° hyphae by 88.1 %. Thus the oil seemed to have exerted a fungitoxic effect on the spore via the hyphae.

The powdery mildew diseases of vine and blackcurrant were controlled to the same degree as for cucumber mildew. Fraction 22, however, was shown in Section I to be heavily phytotoxic when sprayed onto vine leaf discs at the 0.15 ml level. Even when diluted to 60 % in BP85 it still produced an average of 6.7 necrotic or oil soaked spots/discs.

In conclusion, it is clear that phytotoxicity and fungitoxicity of (A + H) components are too closely associated for the components to be used commercially. The separation of the oil into separate compounds or compound types, as illustrated for fraction 21 in Section I, would be a necessary step in any attempt to determine whether the active principle(s) was also phytotoxic. The facilities for such a separation and the necessary screening procedure were not available at the time.

EXPERIMENTAL SECTION IV

THE EFFECT OF OIL ON CHOCOLATE SPOT DISEASE OF BEAN (VICIA FABA L.)

INTRODUCTION

Mineral oils retard the development of leaf spot diseases caused by Cercospora sp. on banana (Guyot and Cuille, 1955), citrus (Fisher, 1961), tung (Kilby et al.,1961) and sugar beet (Schneider, 1965), but have no measurable effect on those on tobacco, peanut, zinnia or gramolote grass (Calpouzos et al., 1962). Where available, the evidence suggests that the oil controls the fungus after it invades the host although no mechanism for this has been determined. Thus the effect of oils on plant diseases seems to depend on the specific characteristics of the host/parasite relationship rather than on the fungal genus. Manning (1968) suggested that the effect of oil on Sigatoka leaf spot of banana might be to induce changes in the host plant that lead to the production of phytoalexin-like substances, which act against the pathogen and so control the disease. Calpouzos (1963) showed that banana leaf tissue, which was physically wounded, became inhibitory to the growth of the pathogen and other fungi after 48h. Corke and Jordan (1962) found some evidence of injury in oil treated banana leaf tissue which was not visible to the naked eye. It was suggested by Manning (1968) therefore, that under normal disease control conditions the oil is sufficiently phytotoxic to induce fungitoxic compounds without causing too much loss of leaf function, and in this respect it may be significant that increased disease control is paralleled by increased phytotoxicity. The effect of phytotoxic oils on plant cells was studied by Van Overbeek and Blondeau (1954) who proposed that within any hydrocarbon configuration series of increasing molecular size, the smaller molecules would disrupt the plasma membrane more than larger ones. Much less disruption would occur with molecules as large as those in banana spray oils, but it might be sufficient to cause the production of fungitoxic substances.

The evidence for the induction of inhibitory substances by physical damage is controversial, Sondheimer (1957) reported that carrots which had acquired a

bitter off-flavour due to prolonged cold storage after harvest accumulated the compound, 3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin. The same compound was shown by Condon and Kuc (1962) to be produced after inoculation with, and to be fungitoxic to Ceratocystis fimbriata. The production of the compound was not a specific response and microscopical observation indicated that its production may have been proportional to the extent of penetration by the fungus. They also reported, however, that the production of the compound by partly immersing fresh uninoculated root discs in aqueous solution at room temperature was only c. 2% of that initiated by inoculation with C.fimbriata. Cold temperature treatment was also used by Bell (1967) to produce fungitoxins in the wound sap of Phaseolus lunatus. Leaves and stems were frozen to -20°C and then the sap collected, thawed, and incubated at 20°C. Ether-extractable, ultra-violet-absorbing compounds accumulated rapidly and after 10 min accumulation was more than 80% complete, with little or none accumulating after 30 min. At the same time there was a rapid, corresponding increase in toxicity of the wound sap to Uromyces phaseoli . Similar, though smaller accumulations were produced by comminution of the leaves and stems with water in a blender with filtration of the resultant liquid and subsequent incubation. Passing the material through a food grinder produced accumulations similar to those produced by freezing. Akazawa et al, (1960) showed that sweet potato roots produced the phytoalexin ipomeamarone as a result of injury by the weevil, Cylas formicarius elegantulus. Two coumarin derivatives which had also been observed in fungal infected tissue were also produced. Ipomeamarone was produced in the healthy tissues which had turned brown around the tunnel-like cavities produced by the weevil. The yield, however, was less than that from fungal infected tissue. Cellular damage to bean (Vicia faba) was shown by Deverall and Vessey (1969) to cause the production of the phytoalexin, wyerone acid. Leaves were injured by pin pricks, a wooden rod (4g) dropped from a height of 3 cm, and a metal rod (11.4g) dropped from a height of 10 cm. Bio-assays of the ether soluble acid extracts of the leaves showed that phytoalexin was induced, with highest yields from leaves showing brown marks after damage by the wooden rod. These extracts gave a 78.3% reduction in

germ-tube growth of Botrytis cinerea as compared with growth in control water phase of extracts. The metal rod caused severe damage visible as transparent areas and extracts of this tissue gave only a 47.8% reduction suggesting that many of the cells were killed, thus preventing them from making an active response to damage. Extracts of pin pricked tissue gave only a 17.4% reduction, very little browning having been visible in the tissue. The same report showed that on infection of leaves by B.cinerea extracts of the tissue beneath the inoculation drop also gave a 78.7% reduction in germ-tube growth of B.cinerea as compared with extracts of healthy tissue. The latter result was, however, determined in a different experiment from that concerned with injury, and thus the sensitivity of the fungus to the extracts may have varied. No quantitative measurements of wyerone acid were made. In contrast, Cruickshank and Perrin (1963,1971) found no difference in the production of the phytoalexins pisatin and phaseollin between endocarp tissue of pea (Pisum sativum) and bean (Phaseolus vulgaris) respectively, which had been sporified in situ., and control tissue. In many of these reports, however, rather crude methods were used to produce wounding, and it may be that the oil, after it penetrates the leaf tissue, might come into such intimate contact with plant cells as to be capable of producing high levels of phytoalexin without showing any signs of visible injury.

Chocolate spot of bean (Vicia faba) is an example of a disease in which the role of the phytoalexin, wyerone acid, has been studied (Deverall and Vessey, 1969; Letcher et al., 1970; Mansfield, 1972) and one in which there is evidence, as described above, for the induction of the phytoalexin by physical damage. It was thus a convenient disease with which to test the above hypothesis. However, initial experiments which were carried out to determine the effect of BP85 oil on the disease showed that the lesions spread more rapidly on oil treated leaves than on control leaves. Later experiments were therefore carried out to investigate the effect of the oil in increasing the spread of the disease rather than in controlling it.

RESULTS

A. The Effect of BP85 on Chocolate Spot

The phytoalexin, wyerone acid, is produced in Bean leaves on infection by both Botytis fabae and B.cinerea. B.fabae, however is less sensitive and is able to metabolize the phytoalexin and so cause lesion spread whereas B.cinerea is more sensitive, unable to metabolize it and rarely produces spreading lesions (Mansfield, 1972). According to the proposed hypothesis therefore the oil would induce the production of larger amounts of phytoalexin than normal, and thus reduce the rate of lesion formation and spread by B.fabae. The hypothesis was tested in the following experiments.

1. The effect on lesion production and spread by B.fabae

Detached leaves were sprayed with BP85 at levels of 0.4 and 0.8 ml as described previously, and left for 20 and 68h before inoculation to allow the oil time to act on leaf. Inoculations were carried out as usual and lesion formation accorded at 24h after inoculation. Lesion spread was observed at 24, 48 and 96h and graded as a, b or c as described previously. It was noted that lesions dried more rapidly on oil treated leaves and this was also recorded. The results are shown in Tables 80 and 81 with representative leaves illustrated in Plate 9.

Table 80 The effect of BP85 on lesion formation by B. fabae

Percentage of inoculations producing lesions				
Pre-inoculation spray time (h)	Oil level (ml)			
	0.4		0.8	
	Control	Oil	Control	Oil
20	97	100	100	100
68	97	98	97	98

Table 80 cont.

Pre-inoculation spray time (h)	Percentage of dry inoculation drops: Oil level (ml) 0.4 Control	0.4 Oil	0.8 Control	0.8 Oil
20	3	7	5	3
68	0	15	0	70

Table 81 The effect of BP85 on spread of lesions of B.fabae

(Percentage of lesions in grades)

Pre-inoculation spray time (h)	0.4 Control a	b	c	0.4 Oil a	b	c	0.8 Control a	b	c	0.8 Oil a	b	c
24h incubation												
	Lesion spread grades											
20	7	0	0	70	0	0	3	0	0	57	30	0
68	20	0	0	78	17	0	13	0	0	5	65	7
48h incubation												
20	88	0	0	17	85	0	85	0	0	20	82	0
68	92	0	0	13	87	0	73	0	0	5	85	0

Table 81 cont.

96h incubation									
20	2	98	0	0	70	30	0	93 70	0 37 63
68	0	95	5	2	65	30	5	88 0	0 42 58

Discussion

The oil treatments had no significant effect on lesion production although with the 68 h pre-treatment a significant number of inoculation drops had dried. This was much more marked at the 0.8 ml level than at the 0.4 ml level. It would seem likely, therefore, that either the oil per se. or the oil in combination with the fungus, had increased the permeability of the leaf so that the water was absorbed by the leaf.

Table 81 and Plate 9 clearly show that the effect of the oil on the disease was to increase the spread of lesions. Although by 6 d there were no clear differences between control and oil treated leaves, on leaves where all the lesions had coalesced the area of the lesions were clearly visible on control tissue, whereas on oil treated tissue they were completely indistinguishable from the rest of the blackened tissue. Thus, when measured at 24 h or later the oil treatments did not reduce the rate of lesion formation or spread. It was possible, however, that the oil may have had an effect at a much earlier stage of lesion formation. This was studied in the following experiment together with the effect of lower spore concentrations.

2. The effect of BP85 on lesion formation using low spore concentrations

Leaves were sprayed with BP85 at levels of 0.1 and 0.3 ml, as the previous experiment showed that higher levels increased lesion spread. The oil was left on the leaves for 48 h when they were inoculated with B.fabae at concentrations of 5×10^{3} and 5×10^{4} spores/ml using 2 µl drops and ten drops per half-leaflet. The effects of the two spore concentrations were compared on the same leaf whilst

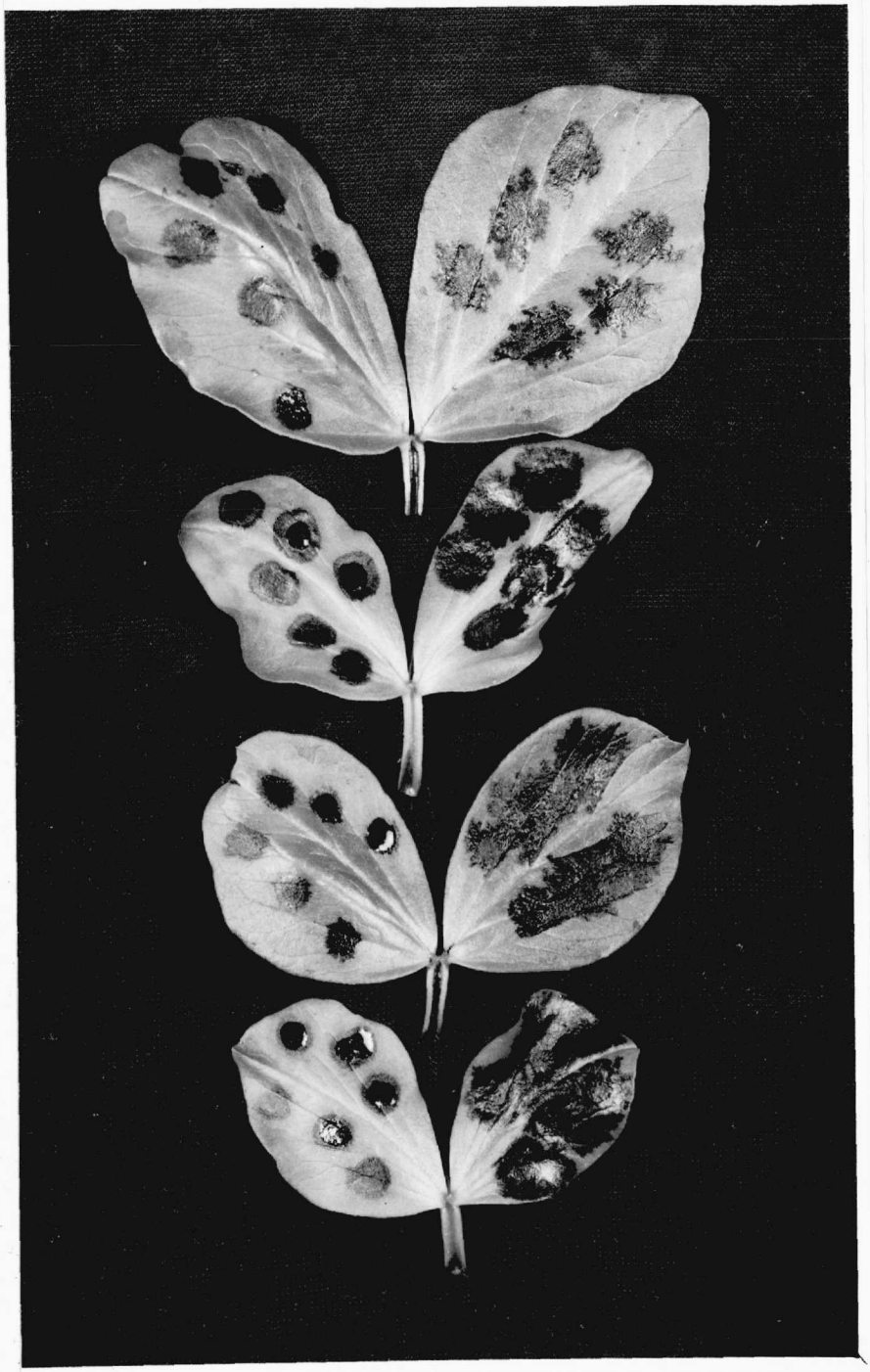


PLATE 9 Effect of BP85 on lesion spread by B.fabae (R.H. leaflets sprayed with oil; L.H. leaflets unsprayed; 2nd from top and bottom pair are No.1 (oldest) leaves; top and 3rd from top are No.3 leaves)

the effects of the two oil levels, applied either to the upper or lower leaf surface, were compared on separate leaves. 40 inoculations were used for each treatment Lesion formation was greaded according to the scale from C to S_3 illustrated in Fig.4 and recorded at 10, 16, 22, 34, 58 and 82 h after inoculation. The appearance of lesions were recorded both on the upper and lower leaf surfaces. Results are shown in Table 82 and Figs. 33, 34 and 35.

Table 82 The effect of BP85 on lesion formation at low spore concentrations and oil levels (Percentage of inoculations in grades)

Lesion grades	Time after inoculation (h)											
	10		16		22		34		58		82	
	C*	O**	C	O	C	O	C	O	C	O	C	O
5×10^4 spores/ml; upper surface spray; 0.1 ml oil level												
F+	75	53	68	70	50	30	10	8	5	0	5	0
F++	18	20	30	23	50	55	33	8	10	3	15	0
F+++	0	0	0	0	0	3	55	68	65	45	45	30
S_1	0	0	0	0	0	0	3	10	20	53	35	70
5×10^3 spores/ml; upper surface spray; 0.1 ml oil level												
F+	30	33	58	55	60	65	45	25	-	-	-	-
F++	0	0	0	0	3	0	15	23	-	-	-	-
F+++	0	0	0	0	0	0	8	20	-	-	-	-
5×10^4 spores/ml; upper surface spray; 0.3 ml												
F+	50	50	53	58	33	38	13	15	0	0	0	0
F++	18	15	30	25	48	40	5	10	10	3	5	0
F+++	0	0	0	0	5	15	65	43	70	30	85	25
S_1	0	0	0	0	0	0	0	25	15	68	5	75

Fig. 33 The effect of BP85 on lesion formation by B.fabae

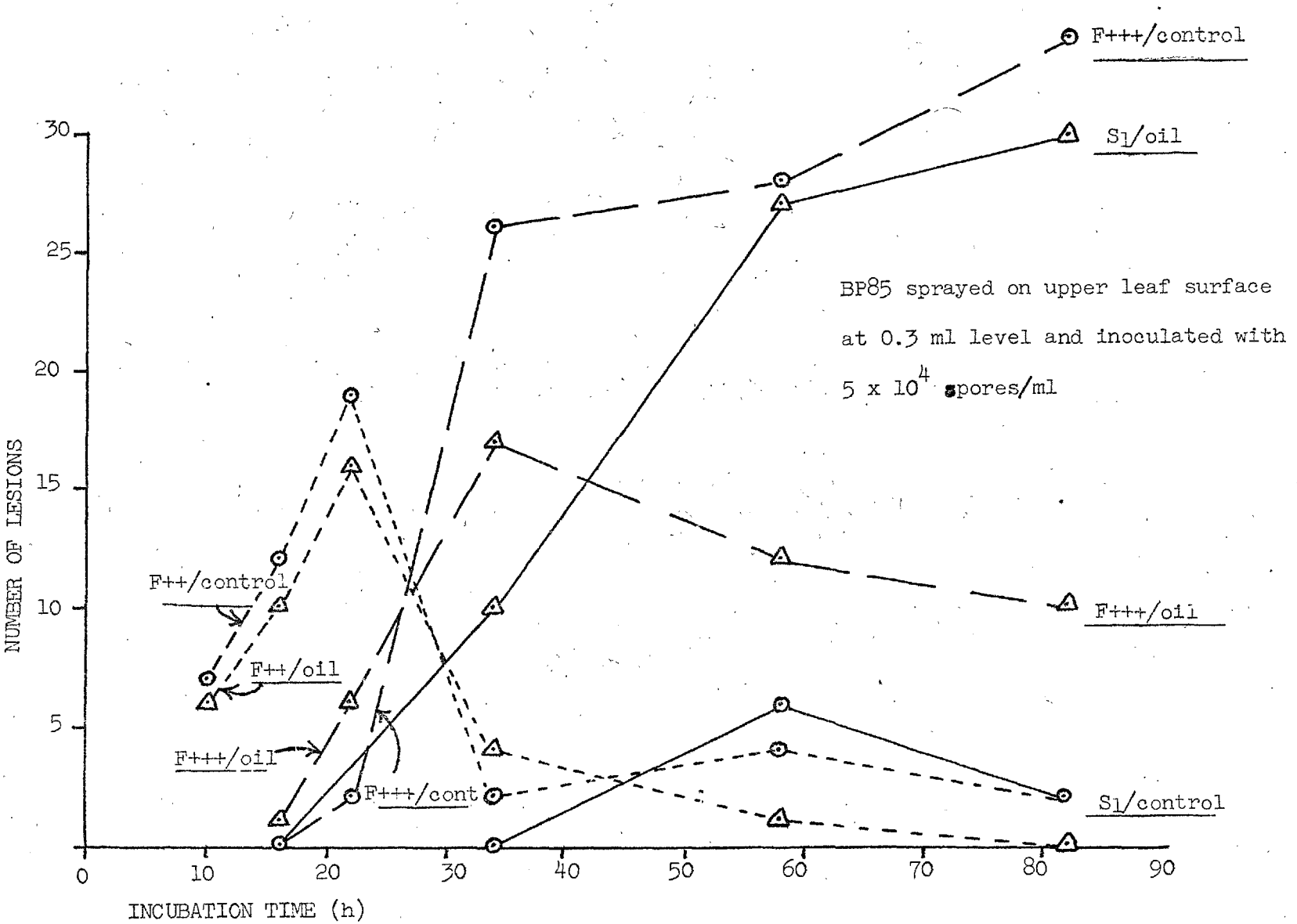


Fig. 34 The effect of BP85* sprayed on upper leaf surface on lesion formation** on both surfaces

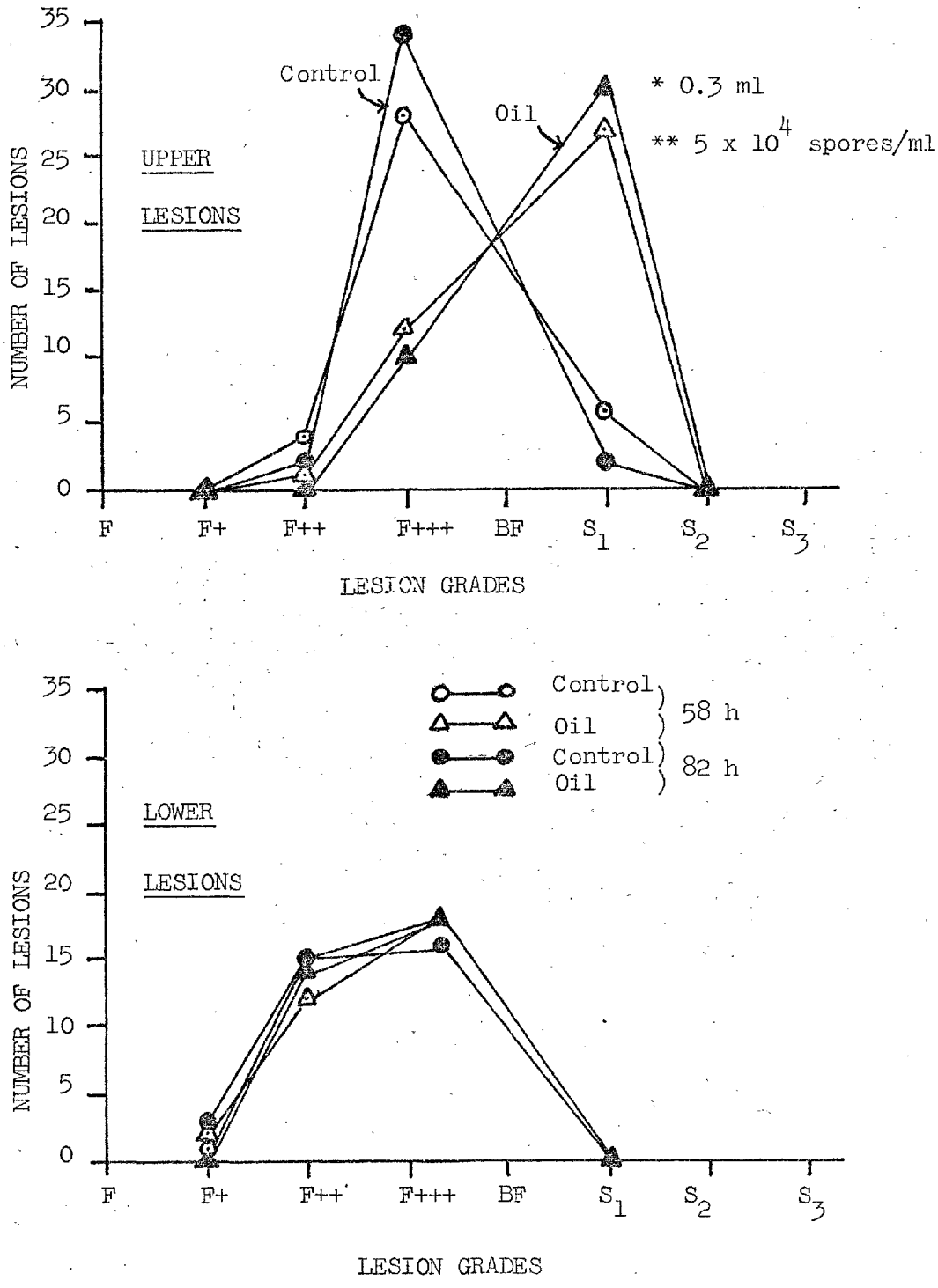


Fig. 35 The effect of BP85* sprayed on lower surface on lesion formation** on both sides

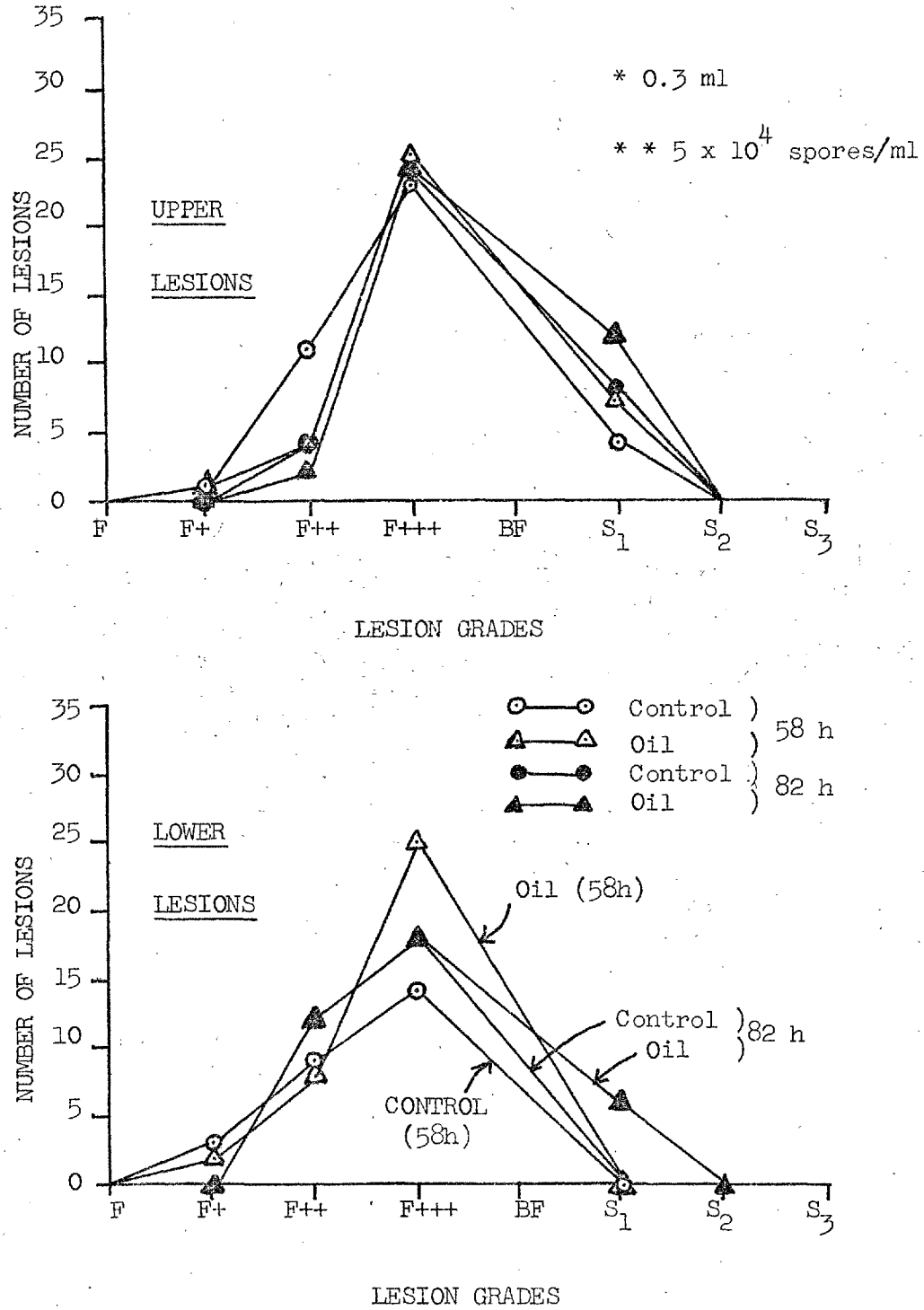


Table 82 cont.

5×10^3 spores/ml; upper surface spray; 0.3 ml												
F+	40	25	60	43	58	50	15	13	-	-	-	-
F++	0	0	0	0	5	0	35	23	-	-	-	-
F+++	0	0	0	0	0	0	18	28	-	-	-	-

* C = control; **O = Oil

Discussion

Even with the low spore concentrations used, BP85 did not produce any significant inhibition of lesion development. The most noticeable effect of the experiment was the increase in lesion development at the highest inoculum level (5×10^4 spores/ml) and oil level (0.3 ml) used as shown in Fig. 33. There was no significant difference between controls and oil treatments for F+ and F++ lesions or for F+++ lesions up to 22 h. At 34 h there was a larger percentage of F+++ lesions on control leaves than on oil treated leaves whereas there was a significantly higher percentage of S_1 lesions on oil treated leaves than on control leaves. At 58 h the difference in F+++ lesions was also significant with the difference in S_1 lesions increased, these results being even greater at 82 h. This is clearly shown in Fig. 34 which also shows that the oil had no effect on the appearance of lesions on the lower leaf surface. This localization of the effect of oil to the surface to which it was applied was also shown, though to a lesser degree, by applying oil to the lower surface and inoculating the upper surface, the results being shown in Fig. 35. This effect was studied further in the next experiment.

3. A comparison of the effect of oil applied to the upper and lower leaf surfaces

Leaves were sprayed with BP85 at the 0.8 ml level to ensure a large effect. Leaves were sprayed either on their upper or lower surfaces with, in each case, the upper surface being inoculated with 10 x 5 µl drops of a suspension of 5×10^5 B.fabae spores/ml. Lesion development was observed at 24 and 48 h.

Lesion formation was difficult to assess using the grading system due to the irregularity of the drying of inoculation drops and the irregular nature of the lesions. At 24 h many of the oil sprayed leaves were heavily transluscent in the area of the inoculation drops even through there was no obvious darkening of the tissue. Leaves with oil applied to their upper surfaces showed much more lesion spreading than the controls, but lesion development through to the lower surface of oil treated leaves was slightly less than on control leaves. Leaves with oil applied to the lower surface showed very clear transluscent areas on the lower surface when held over a light background. These areas appeared almost black when viewed without any lighting from the lower surface, and yellow when viewed from the upper surface. They eventually turned brown and finally black, and would seem likely to have been the cause of the spreading on oil treated leaves recorded in previous experiments.

In all replicates it was clear that only when the oil was applied to the lower leaf surface were the translucent areas visible on that surface. It was also clear that inoculation drops dried more rapidly on oil treated leaves than on control leaves as found previously. This was more pronounced when the oil was applied to the lower surface than when applied to the upper surface.

By 48 h the increased spreading of lesions on oil sprayed leaves was very clear and although there was much more infection on the upper surfaces of leaves sprayed with oil on the same surfaces than on control leaves, there was no difference in lesion development through to lower leaf surfaces between oil treated and control leaves. The blackening of the lower leaf surfaces of leaves infected on their upper surfaces, but sprayed on their lower surfaces was again very clear.

The translucent areas were clearly produced by the oil in combination with the fungus, as neither the infection controls or water drops on oil treated leaves produced these symptoms.

4. Effect on infection by B.cinerea

Previous experiments showed that BP85 increased the rate of development by B.fabae lesions, though by 6 d there were no significant differences between

control and oil treated tissue. As B.cinerea does not normally produce spreading lesions it was of interest to see whether BP85 would cause this fungus to spread. In this case the difference between control and oil treated tissue should be maintained indefinitely.

Leaves were sprayed with BP85 at the 0.4 and 0.8 ml levels on their upper surfaces. After 60 h each leaflet was inoculated with 10 x 5 µl drops of a suspension of B. cinerea spores (5×10^5/ml) as before. Lesion development was recorded on both upper and lower surfaces at 48 h and on the upper surfaces at 5 d. Results are shown in Table 83.

Table 83 Lesion development by B.cinerea on oil sprayed and untreated leaves at 48 h and 5 d

(Percentage of inoculations in grades)

Treatment	Infection grades								
	C	F+	F++	F+++	S_1	S_2	S_3	S^o	S*
48 h - Upper surface									
0.4 ml oil	32	8	2	30	28				
untreated	38	8	20	30	4				
0.8 ml oil	14	12	6	18	42	8			
untreated	2	38	20	38	2	0			
48 h - Lower surface									
0.4 ml oil	62	0	36	2					
untreated	84	14	2	0					
0.8 ml oil	32	12	34	22					
untreated	84	10	6	0					

Table 83 cont.

5 d - Upper surface									
0.4 ml oil	26	6	2	0	2	4	12	36	12
untreated	20	18	18	34	10	0	0	0	0
0.8 ml oil	0	0	2	0	0	0	0	62	36
untreated	8	0	20	40	12	12	0	8	0

Discussion

By 48 h a significantly greater number of B.cinerea lesions had spread on oil treated leaflets than on control leaflets. That this was true spread and not caused by spreading of infection drops was shown by observations on the lower surfaces of leaves. Lesion development through the oil sprayed leaflets and onto the lower surfaces was significantly greater than through control leaflets.

By 5 d a few more B.cinerea lesions had spread on control leaflets but a significantly larger number of lesions on oil treated leaflets had spread and coalesced so that almost the entire leaflet was blackened. Also, a large proportion of the oil sprayed leaflets showed considerable oil soaking in and between inoculated areas.

B. The Effect of (A + H) Components of Oil Fractions on Chocolate Spot

In Section I and II it was shown that the (A + H) components of experimental oil fractions were the most effective types of oils tested for the control of powdery mildew diseases and downy mildew of vine. It was of interest therefore to determine whether these oils would control chocolate spot.

Detached leaves were sprayed with the (A + H) component of fraction 23 at the 0.15, and 0.30 ml levels. They were inoculated with spores of B.fabae and incubated as before. Lesion development was recorded at 15 and 48 h after inoculation, results being given in Table 84.

Table 84 Lesion development by B.fabae on leaves sprayed with Fraction 23 (A + H) Component

(% inoculations in grades)

Treatment	Infection grades									
	C	F	F+	F++	F+++	BF	BC	S_1	S_2	S_3
15 h										
0.15 ml oil	4	62	25	10	0	0	0	0	0	0
untreated	5	54	27	14	0	0	0	0	0	0
0.30 ml oil	5	53	16	23	0	0	0	0	0	0
untreated	7	63	13	17	0	0	0	0	0	0
48 h										
0.15 ml oil	2	0	2	2	0	2	6	81	2	0
untreated	0	0	2	0	0	0	2	87	9	0
0.30 ml oil	0	0	0	5	5	16	0	49	26	0
untreated	11	0	0	0	0	7	2	57	22	0

Discussion

The oil treatments did not produce any significant reduction in lesion development.

SUMMARY AND DISCUSSION

BP85 oil had no effect on lesion production by B.fabae except that a significantly greater number of inoculation drops dried on oil treated leaves than on control leaves. It is suggested that the oil per se., or in combination with the fungus had increased the permeability of bean leaves to water. The rate of lesion development and spread, however, was significantly increased on oil sprayed leaves. Even using low concentrations of 5×10^{4} and 5×10^{3} spores/ml, no significant inhibition of lesion development was found.

The effect of BP85 on lesion development was localized to the surface on which it was sprayed. Thus although oil sprayed on upper leaf surfaces increased the rate of development and spread of lesions, development through the leaf was only increased when the oil was applied to the lower surface and in this case there was no difference in lesion spread on upper leaf surfaces. Leaves sprayed on their under surfaces showed very clear translucent areas when viewed from below whereas when sprayed on their upper surfaces no translucent areas were visible indicating a greater susceptibility of this surface to oil. Translucent areas developed often before lesions had formed and eventually turned brown and finally black. Whether this whole blackened area was due to the presence of the fungus or possibly to fungal metabolites was not determined. It was clear, however, that the initial translucency was due to a combination of the effect of fungus plus oil as neither water drops on oil sprayed leaves or inoculation drops on unsprayed leaves produced this effect.

If any fungitoxic substances had been produced in the leaf by the action of the oil they were either not produced in sufficient quantity and/or too rapidly metabolized by the fungus to retard its development. It may be, however, that the balance between fungus and host is so much in favour of the former, as shown by the rapid development of lesions, that any fungitoxic substances induced by the oil are counteracted by heavy damage to the leaf caused by a combination of fungus plus oil. It was not clear from these experiments whether any undetected damage caused by the oil might have enabled the fungus to penetrate and spread more rapidly, or whether the presence of the fungus, on or penetrating

through the leaf surface, also enabled the oil to penetrate, and then either damaged host cells by its own action or in combination with fungal metabolites. It may be that the oil causes nutrients to be released by increasing leaf permeability. Thus Sol (1968) showed that pretreating bean leaves with decenylsuccinic acid, which increases cell permeability to water, increased the number of lesions produced when inoculated with spores of B.fabae. This increase was partly attributed to an increase in spore germination associated with a greater concentration of sugars and amino acids in leachates. Deverall and Wood (1961) also showed that simple sugars particularly glucose, increased the number of lesions produced by B.fabae on bean leaves. The ability of oils to produce an increase in permeability of plant cells was shown by Van Overbeek and Blondeau (1954). They used isolated beetroot cells to test the effect of pure hydrocarbons on the release of cell pigments. The smaller molecules produced a greater release than the larger ones, but with those present in plant spray oils, very little effect would be expected. Thus the effect of BP85 per se. on bean leaves would be very small and it would seem more likely that the main effect of the oil would be after penetration of the leaf by the fungus.

BP85 also caused lesions of B.cinerea, which normally remain limited to inoculation areas, to spread between these areas and through the leaf to the lower surface. Thus the effect of wyerone acid, which normally restricts the growth of B.cinerea (Mansfield, 1972) had been overcome. It is unlikely that this was due to the release of simple nutrients such as sugars as these have been shown not to cause B.cinerea lesions to spread (Purkayastha and Deverall, 1965). It has been shown, however, that the water soluble extracts of bean pod diffusates do cause lesions of B.cinerea to spread (Mansfield, 1972), although extracts from leaves have not been tested. Further experiments would therefore be necessary to determine whether the oil, after it penetrates the leaf, causes a similar release of substances and whether this release, if it does occur, is mediated either by the oil alone or in combination with the fungus.

GENERAL DISCUSSION

The failure of a highly refined mineral oil such as BP85 to control chocolate spot adds not only to the increasing list of diseases not controlled by oil but also to those diseases whose severity is increased by oil sprays. These are mainly diseases of banana in areas where oil has been used for many years to control Sigatoka leaf spot caused by Mycosphaerella musicola. This disease is being gradually replaced by black leaf streak (BLS) caused by a very virulent mutant of M. musicola, named M.fijiensis (Leach, 1964) against which, oil sprays are far less effective. Spray trials against this disease carried out by Firman and Hoskin (1970) showed that although there were no differences in the yield of the 'plant' crop, between maneb in water, maneb in oil-water emulsion and oil-water emulsion alone, maneb in water gave much better disease control and leaf survival, the oil seeming to have had an adverse effect on fruit quality but not on plant growth. They also suggested that the oil may even increase diseases caused by other fungi eg Haplobasidium musae, Cordana musae, Uromyces musae and Chloridium musae which are sometimes important. Long (1970) found that misting oil sprayed alone, significantly increased both the number and size of Cordana Leaf spots, a maneb-oil-water mixture causing a similar increase in leaf spot size but not in number of infections. Increases in incidence and severity of Cordana Leaf spot and black spot caused by Deightomella torulosa were also reported by Leach (1959) and Meredith (1961,1962) and leaf speckle caused by Chloridium masae (Meredith, 1963). Firman (1971) reported Malayan leaf spot (MLS) caused by Haplobasium musae to be potentially serious in Fiji particularly in locations receiving low sunshine hours, bananas being sprayed with oil liable to severe infections. During spray trials against BLS it completely replaced this disease causing very rapid leaf loss. On plots receiving no oil MLS appeared late and was never severe, BLS remaining the major disease.

Thus in addition to the original reason for changing over from oil alone to oil plus fungicide for control of Sigatoka leaf spot, which was the reduction in yield caused by the oil, and the increased risk of phytotoxicity on young

plants, or plants grown in conditions of drought or near drought, the increased occurrence and severity of other diseases due to oil spraying is beginning to assume some importance. As Meredith (1970) has pointed out, there seems little doubt that for Sigatoka disease control low volume aqueous sprays would have come into general usage had oil sprays not been discovered. At present, however, the disadvantages of using oil have not precluded its use altogether and moreover, it has been found that the effectiveness of the systemic fungicide benomyl is increased when applied as an oil-water emulsion (Long, 1971). In this case the oil seemed to be acting synergistically with benomyl to control the disease.

The symptoms of increased severity of chocolate spot caused by oil sprays described in the present work are similar to those described by Firman (1971) for MLS. Lesions were surrounded by a distinctly water soaked area often several times the size of the lesion and extending parallel to the veins merging with similar areas from adjacent lesions. The inability of oil to control chocolate spot is perhaps not surprising in view of the coincidence pointed out by Calpouzos (1966) between the two diseases Sigatoka leaf spot and greasy spot of citrus caused by Cercospora citri-grisi, both of which are controlled by oil and also have relatively long incubation periods, the relationship between host and pathogen being thus finely balanced. Chocolate spot, however, has no incubation period inside the host leaf, the host - pathogen balance being very much in favour of the latter.

A possible mechanism of action of oil in controlling Sigatoka leaf spot has been discussed previously in relation to both chocolate spot and vine downy mildew. This involved the supposition that the oil induced the production of a phytoalexin or phytoalexin-like compounds which inhibit the growth of the pathogen in the leaf. A detailed study of the action of oil on the life cycle of M.musicola by Stover and Dickson (1968) showed that only in a few cases (20% or less) was pathogenesis completely stopped whilst in the majority of cases the effect of oil was to increase the incubation period and generation time. This does not conflict with the phytoalexin hypothesis as the limited data available suggests that the ability of leaves to produce phytoalexins

decreases as leaves senesce (Bailey, 1969). This provides some circumstantial evidence for the involvment of phytoalexins in the control of vine downy mildew with an unsaturated oil fraction, where control decreased slightly with leaf age. Whether repeated oil applications to banana leaves completely inhibit Sigatoka leaf spot symptoms and thus maintain the ability of old leaves to keep the fungus in check has not been determined.

An interesting fact noted by Stover and Dickson (1968) and perhaps relevant to the control of vine downy mildew with BP85 in field experiments reported herein, was that they consistently obtained a partial inhibition (up to 44%) by oil sprays of germination of spores of M.musicola on leaves in the field, whereas no inhibition was obtained under laboratory conditions. This might partially explain the results obtained with BP85, the wide variation in control from leaf to leaf being paralled by a similar variation in the control of Sigatoka leaf spot.

The results of tests against cucumber powdery mildew show that refined mineral oils such as BP85 are of little use against the disease except at very low inoculum levels. Increased disease control was found for saturated oils with lower distillation temperatures than BP85 but commercial control would only be possible with oils of such low distillation temperatures as to be too volatile and cause too much phytotoxicity to be of any use. Although good control was obtained with unsaturated oil fractions, phytotoxicity was high. This also applied to vine downy mildew, the difference in phytotoxicity between the most active fraction showing minimal phytotoxicity on leaf discs, and on intact leaves in the greenhouse being considerable.

The complexity of oil fractions as shown by mass spectroscopy is very high and it has yet to be determined whether the fungitoxic and phytotoxic properties of these fractions are due to separable constituents.

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APPENDIX I PHYSICAL CONSTANTS OF OILS

A. The (A + H), (N + P) and Wax Content of Experimental Crude Oil Fractions

These were determined as described previously and are shown in Fig. 36 with the (N + P) components given minus their wax contents.

B. Oil Viscosity

1. Wide distillation range oils

The viscosities of gas oil, BP85 and 150 Neutral have been given previously in Table 3 (p.32). Figs.37 and 38 show the variation in viscosity with distillation temperature and variations in the viscosity of each oil with working temperature.

2. Experimental oil fractions

Viscosities of components of oil fractions were determined at 37.8°C as described on p.40, the viscosities of (N + P) components of fraction 22 and 24 being tested minus their wax contents as the was was solid at room temperature. The results are given in Fig. 39 except that there was insufficient (A + H) component of fraction 24 for an accurate determination. A rough estimate was obtained by using half the normal quantity of oil required for the viscometer, and allowing the oil to run about half its length. Multiplying this by two gave a very rough time estimate and thus a rough viscosity estimate of 72 centistokes.

Estimates of the viscosities of the diluted (A + H) components of oil fractions at c. 20°C using the pipette method described previously are shown in Fig. 40.

C. Oil Volatility

The volatility of gas oil, BP85, 150 Neutral were tested together with the (N + P) components of experimental oil fractions 13, 16, 19, 21, 22 and 23 and the (A + H) components of fractions 16, 19, 21, 22 and 23. Determinations were carried out as described on p.40 and volatility, expressed as weight loss, plotted against time in Figs. 41 and 42, and the relationship between percentage volatility and 50% distillation temperature of F.19, 21 and 23 (N + P) components

Fig. 36 The hydrocarbon component content of crude oil fractions

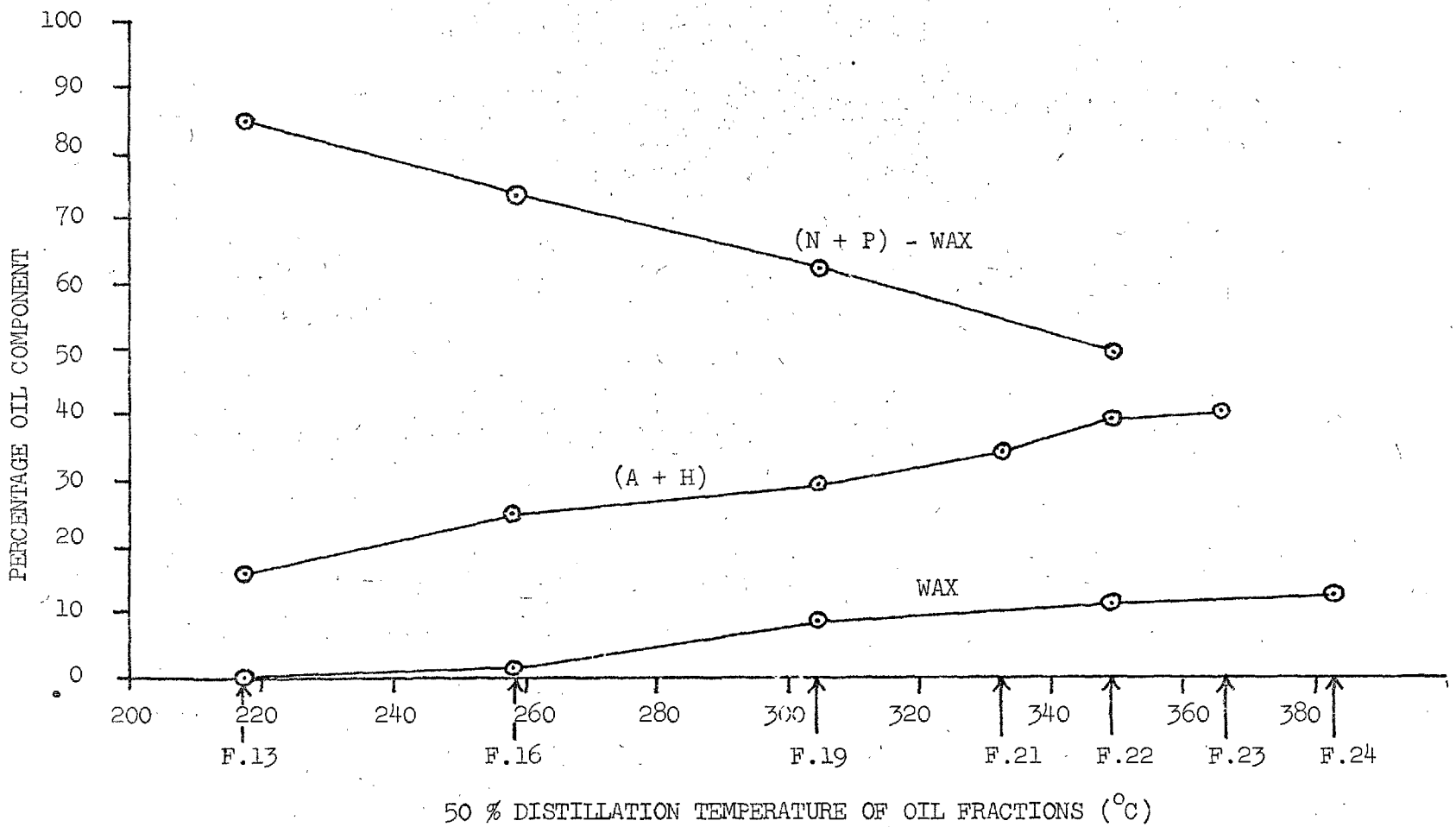


Fig. 37 The variations of viscosity of wide distillation range oils with distillation temperature

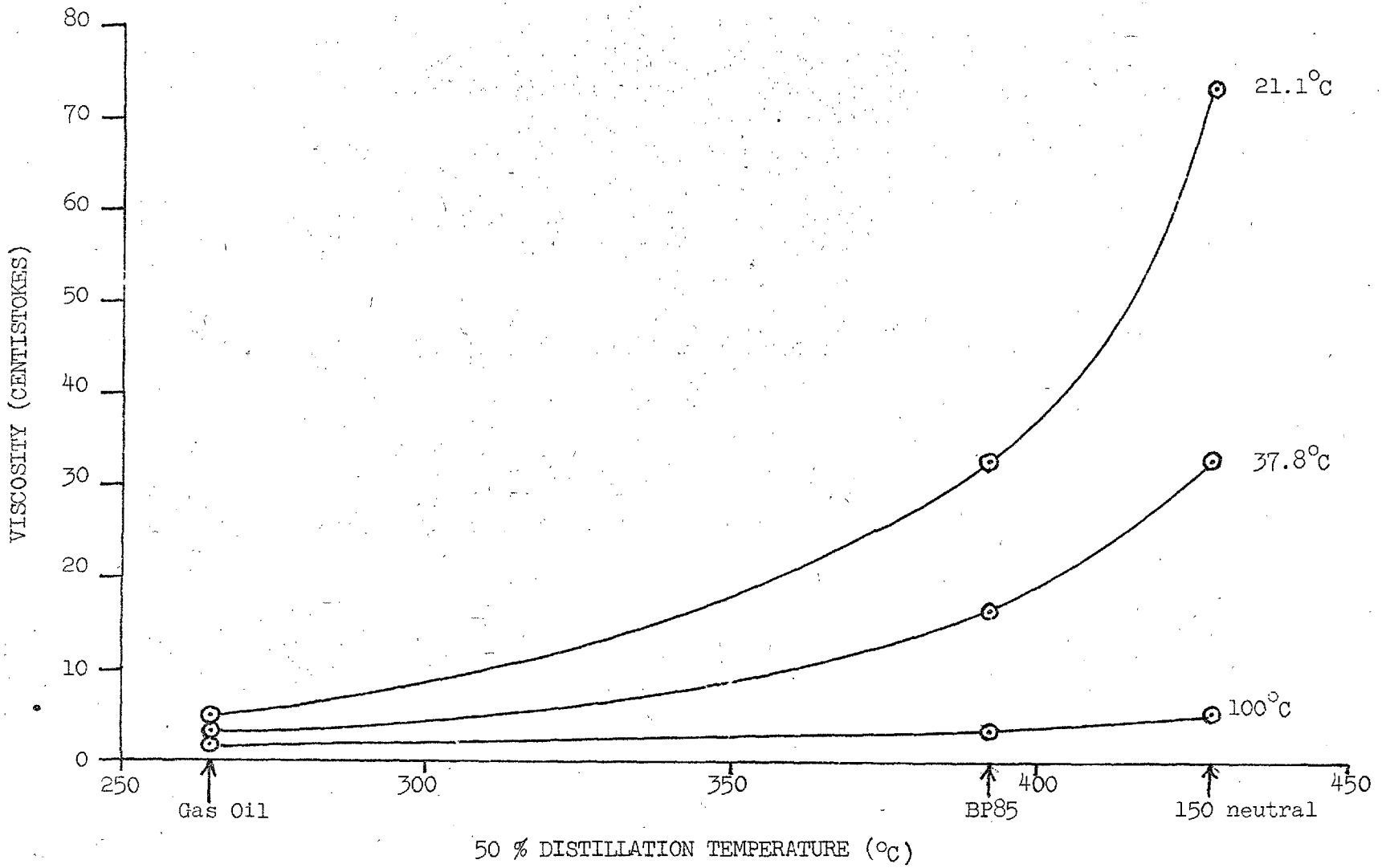


Fig. 38 The variations of viscosity of wide distillation range oils with working temperature

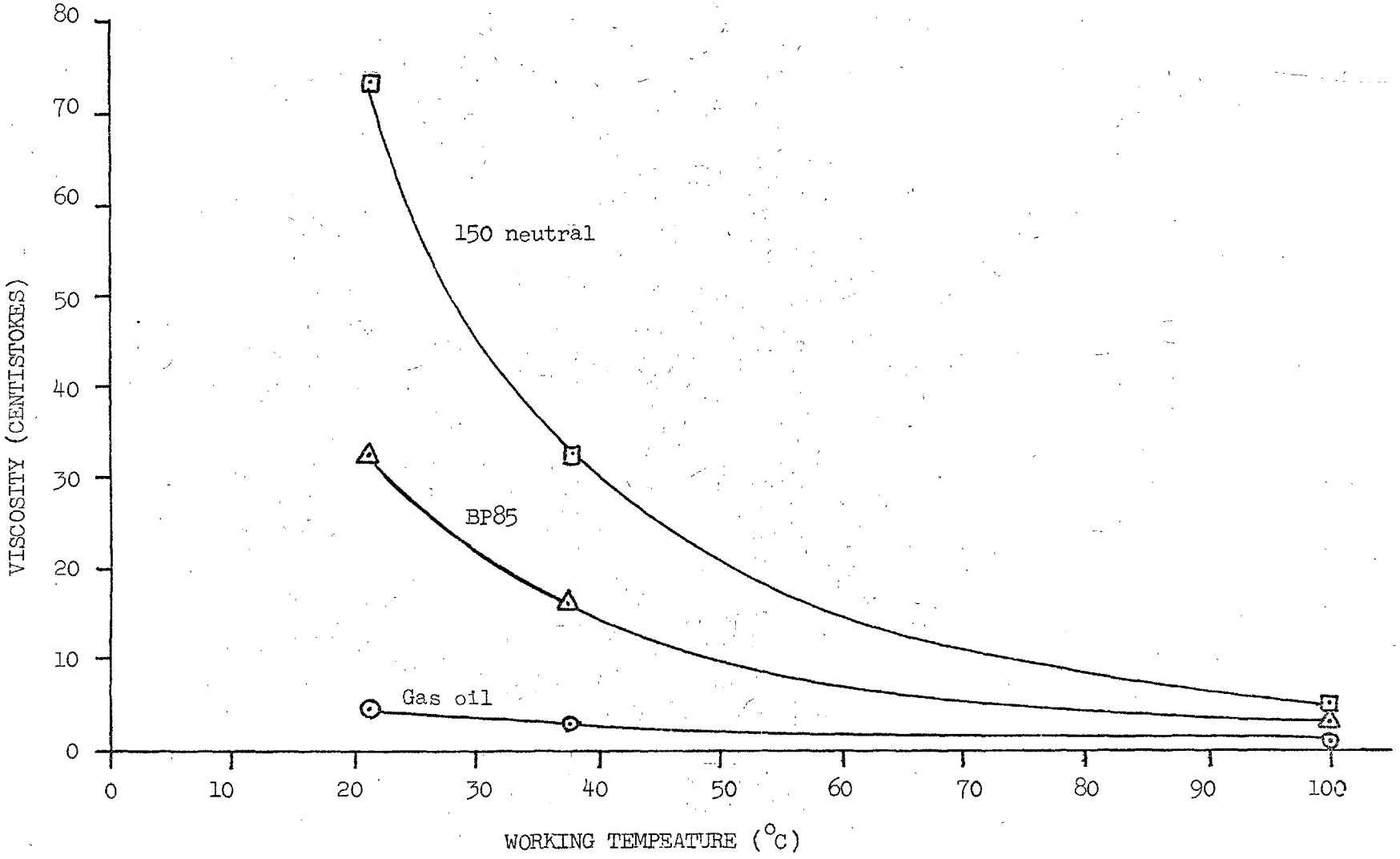


Fig. 39 The viscosities of (N+P) and (A+H) components of experimental oil fractions

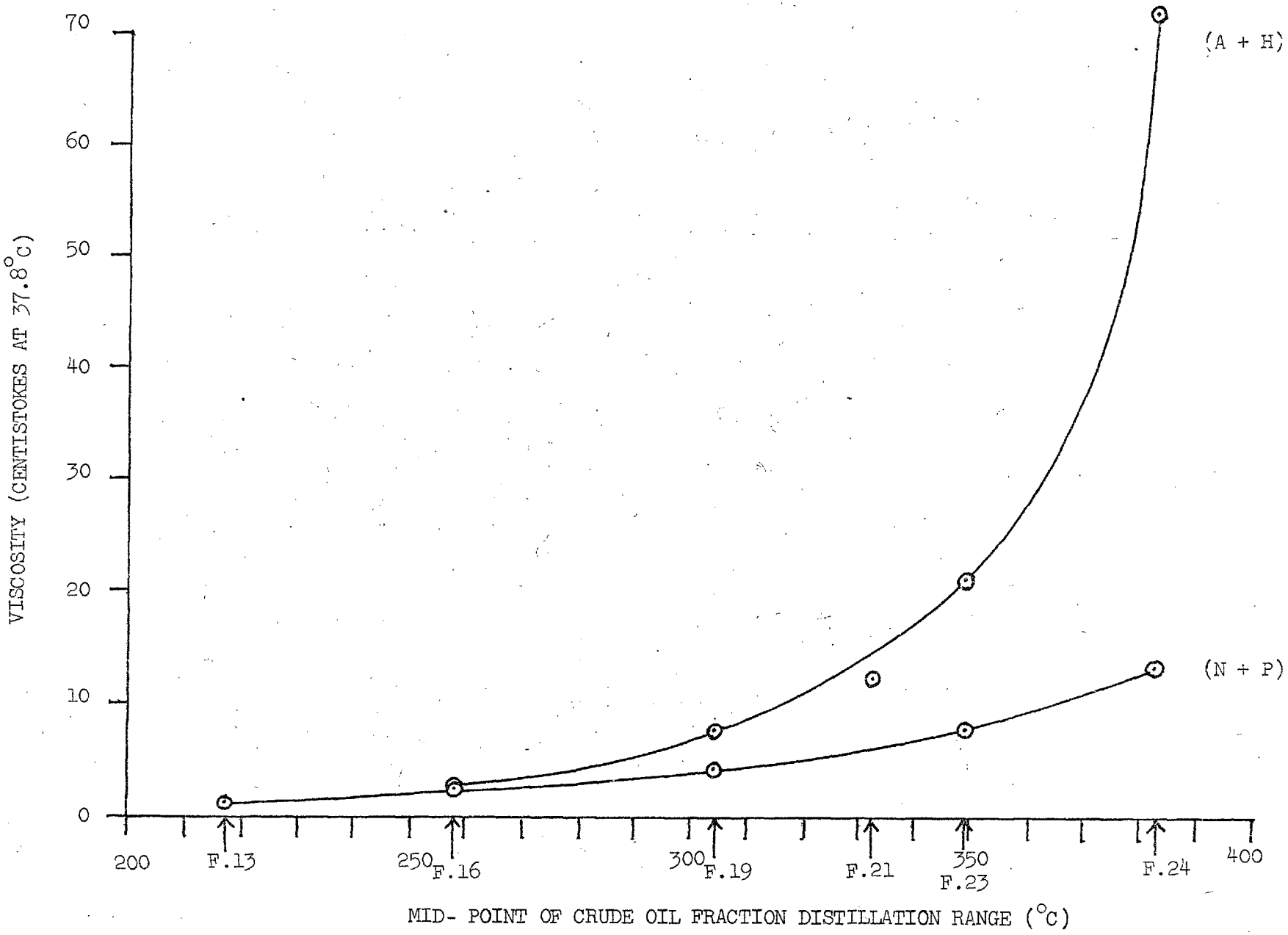


Fig. 40 Viscosities of diluted (A + H) components of experimental oil fractions

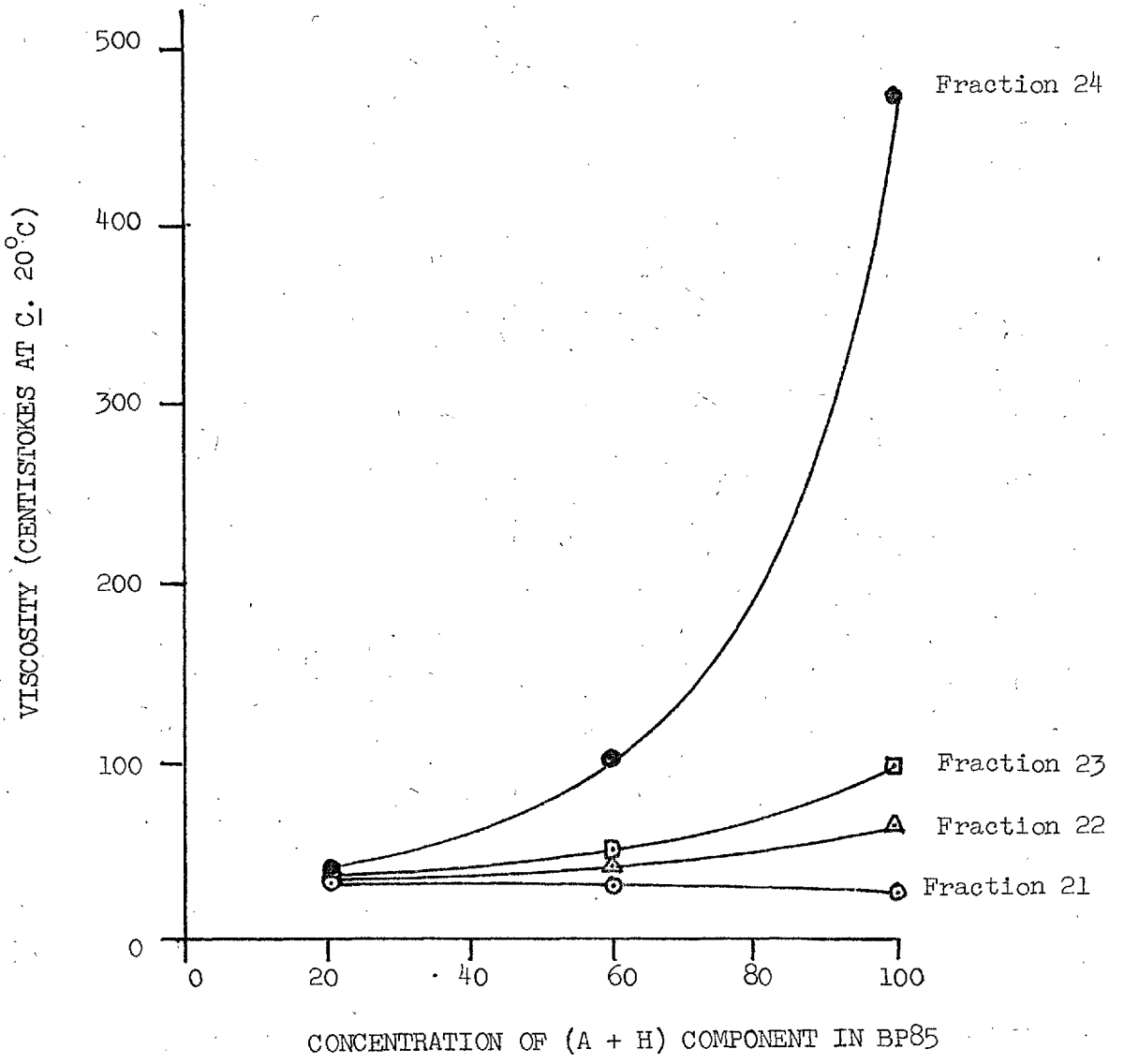


Fig. 41 Volatility of Gas Oil, BP85 and 150 Neutral

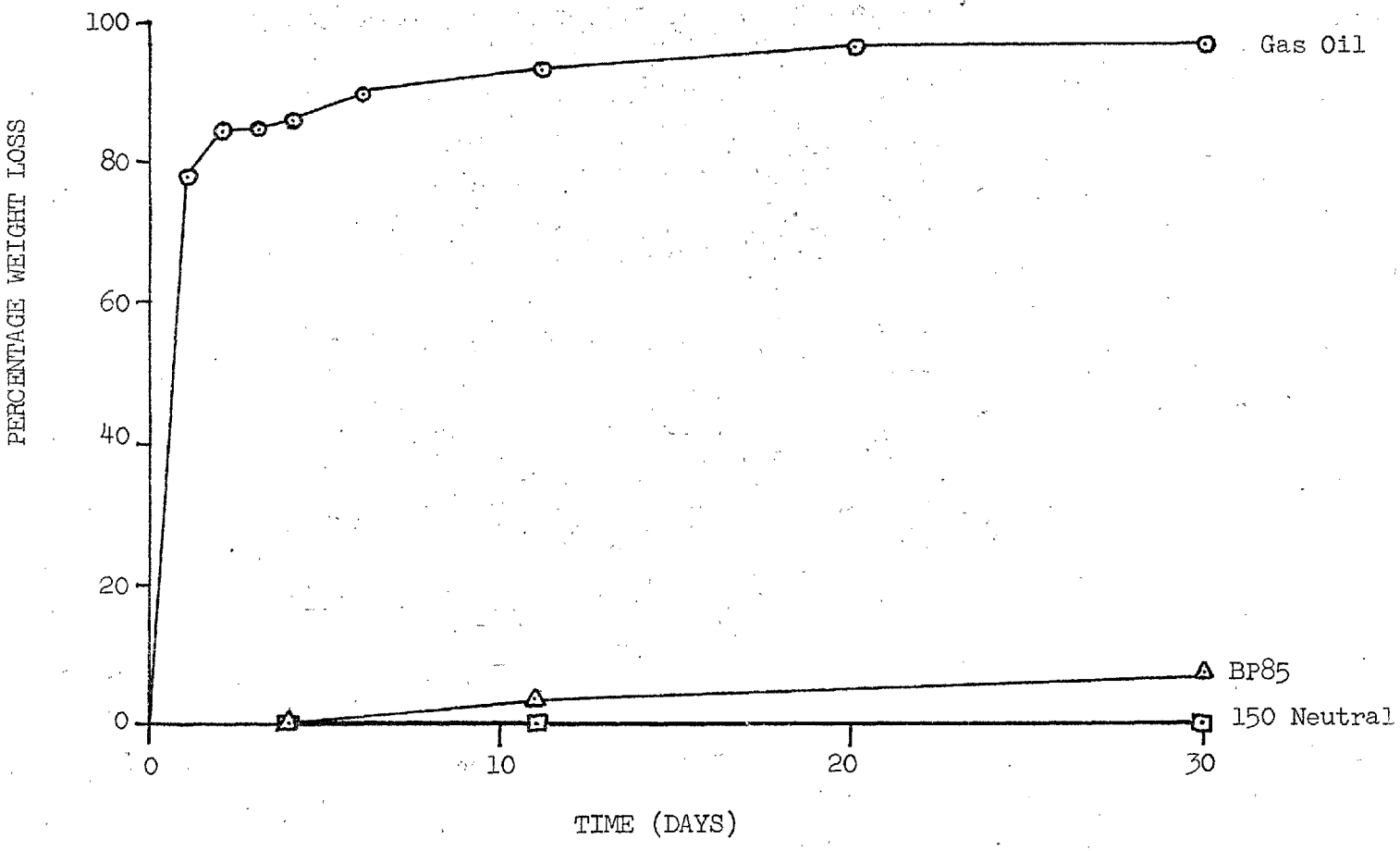


Fig. 42 Volatility of Oil fractions

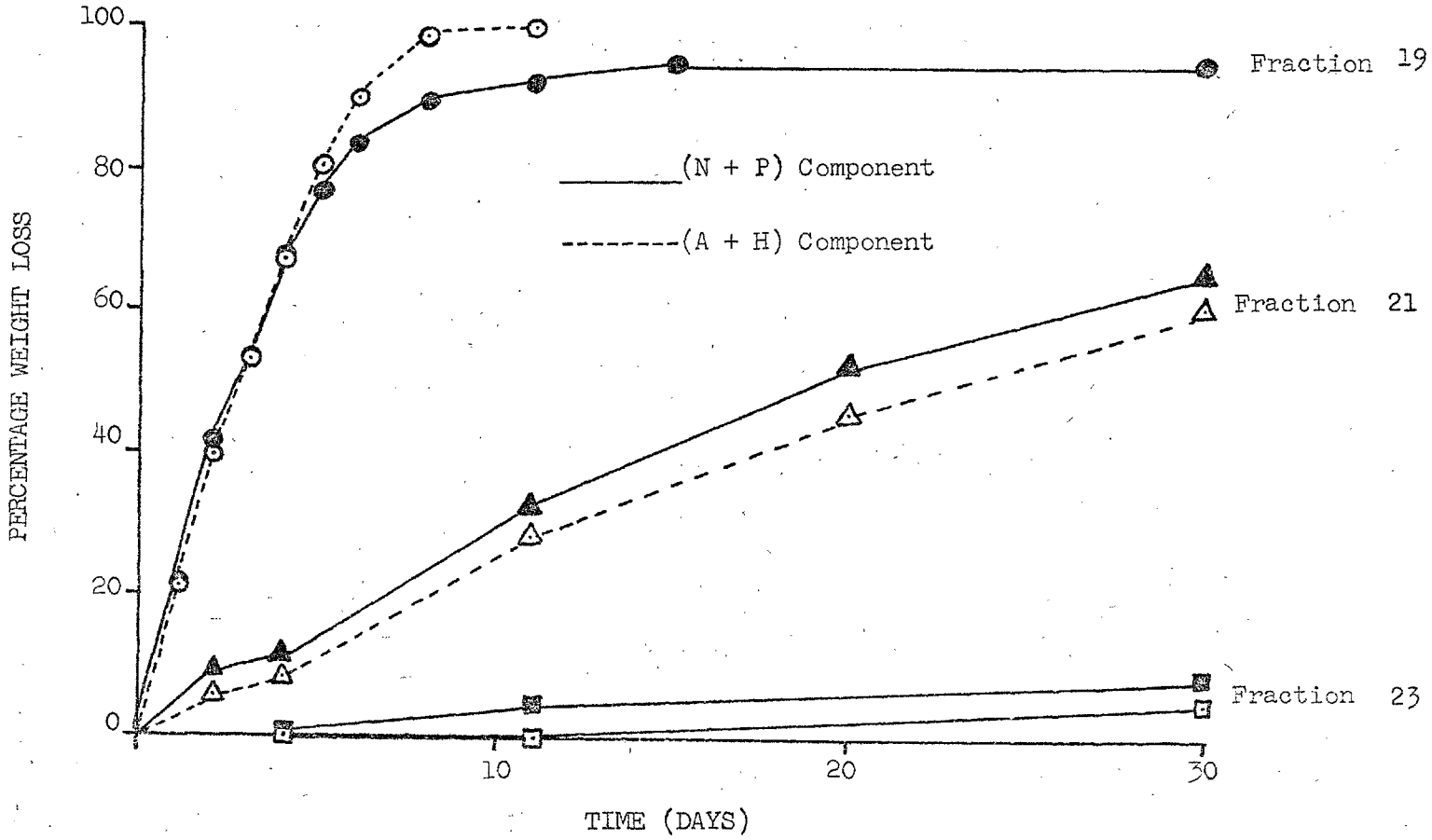


Fig. 43 Relationship between volatility and distillation temperature of F.19, 21 and 23 (N + P) components

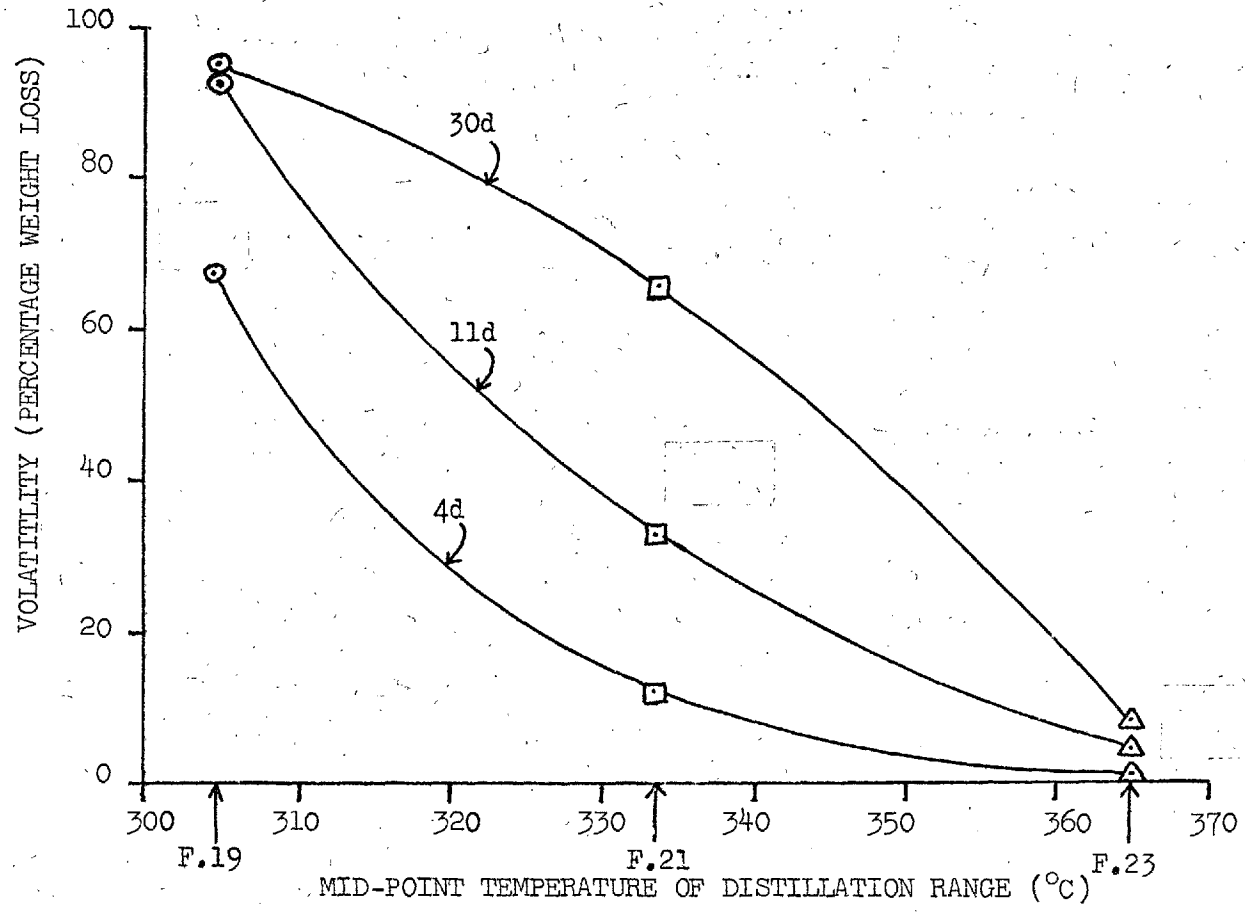


Fig. 44 Potter Tower calibration with BP85 (Side arm method)

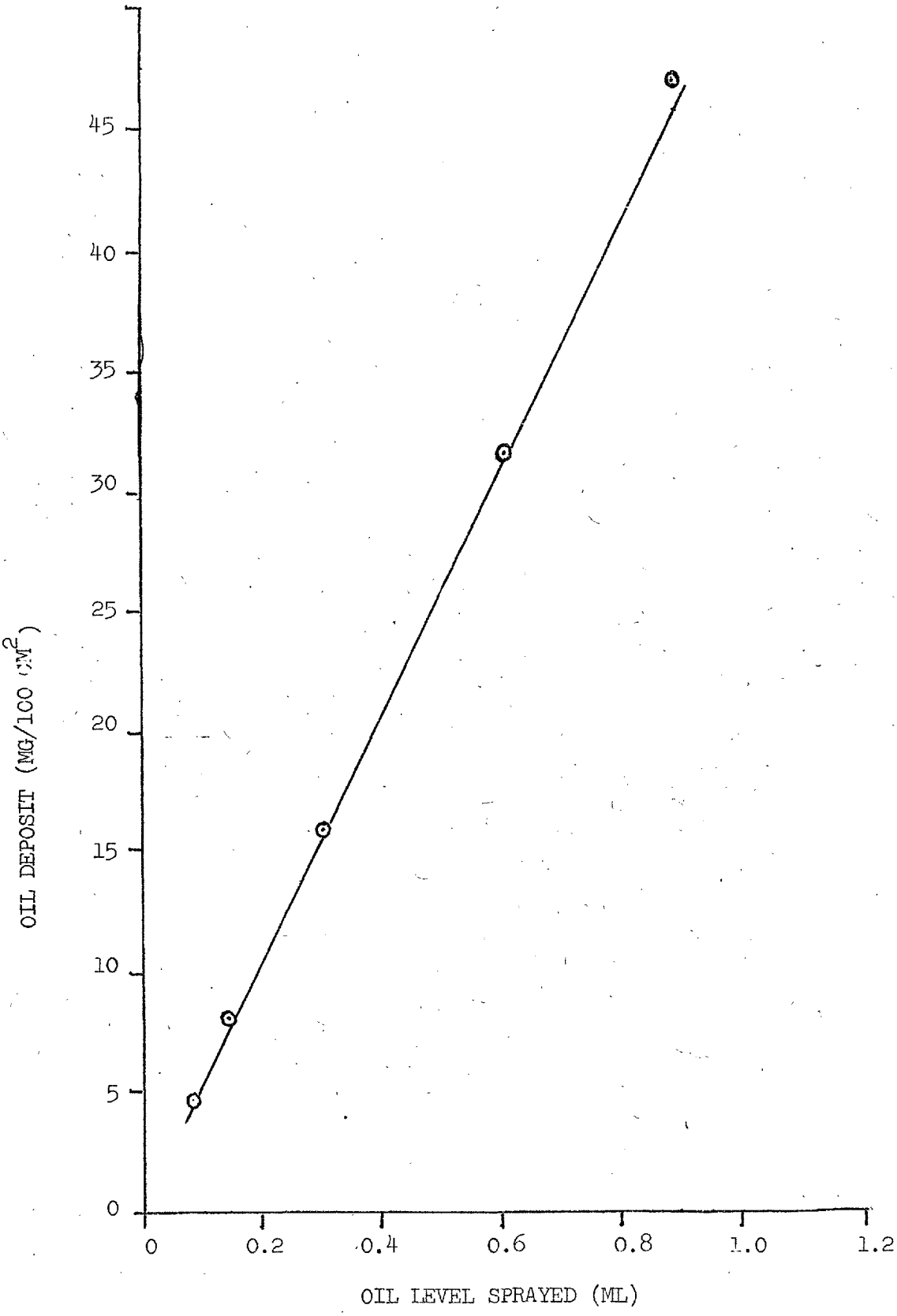


Fig. 45 Potter Tower calibration with (N + P) components of oil fractions (side arm method)

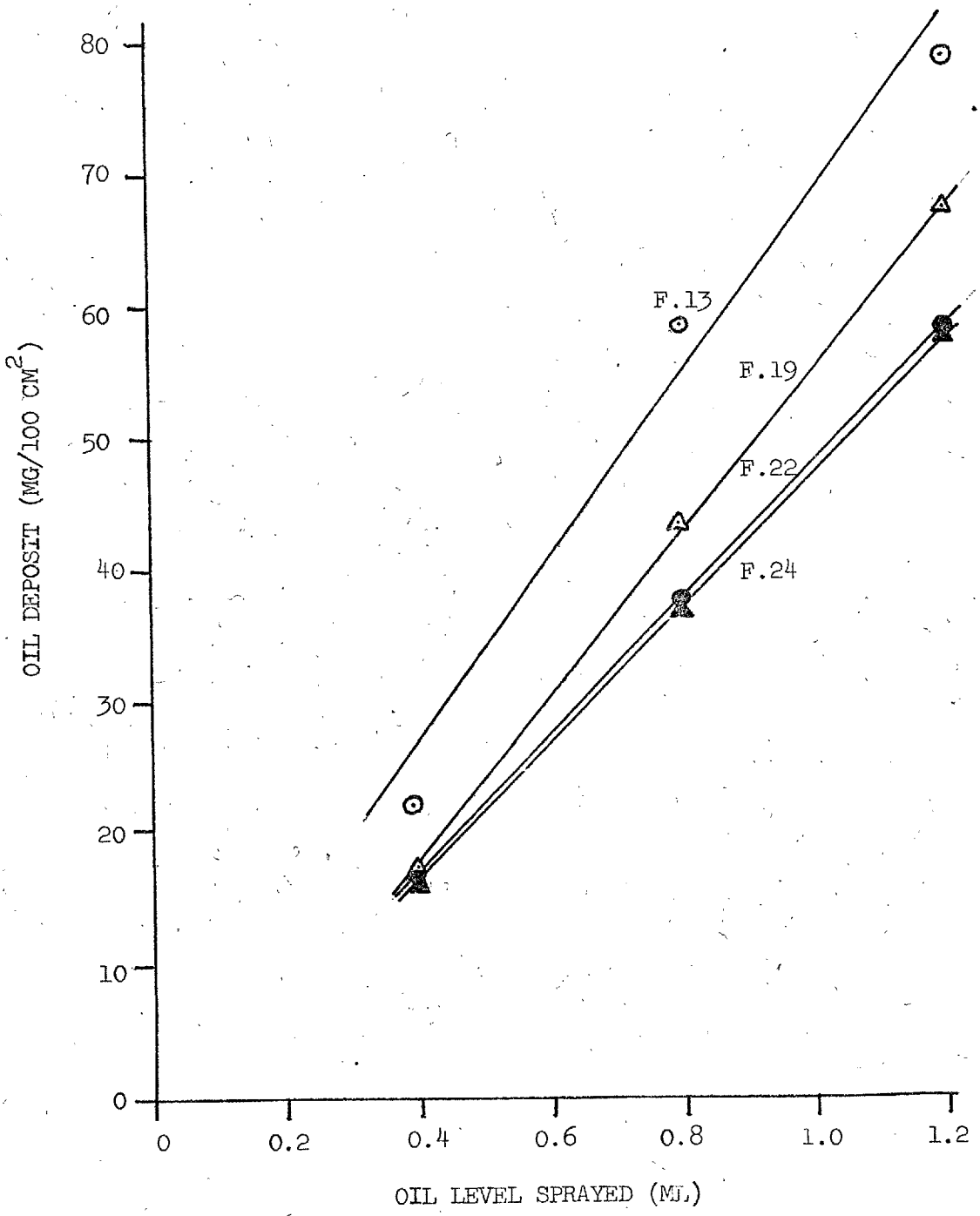


Fig. 46 Potter Tower calibration with (A + H) components of oil fractions (Pipette method)

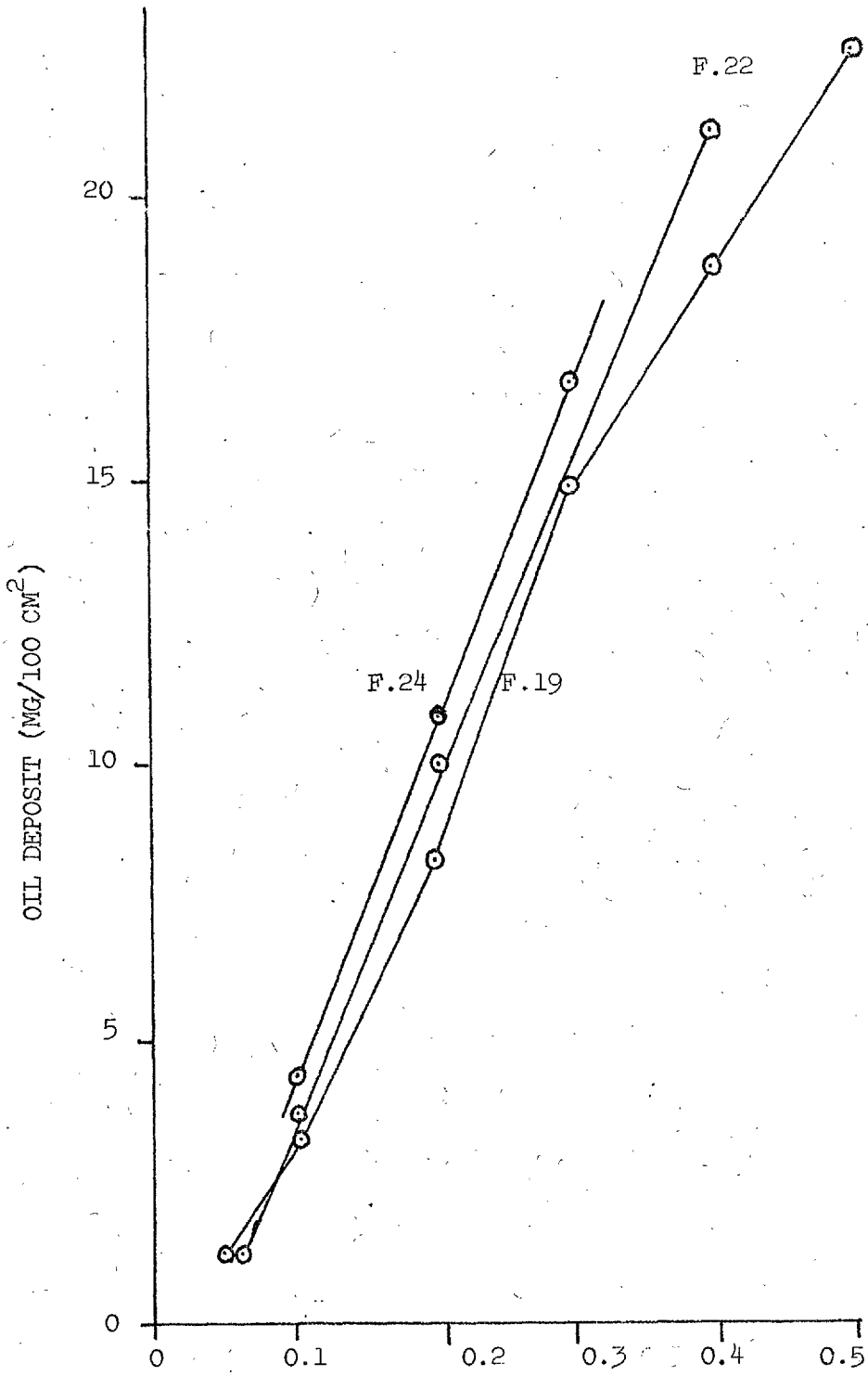
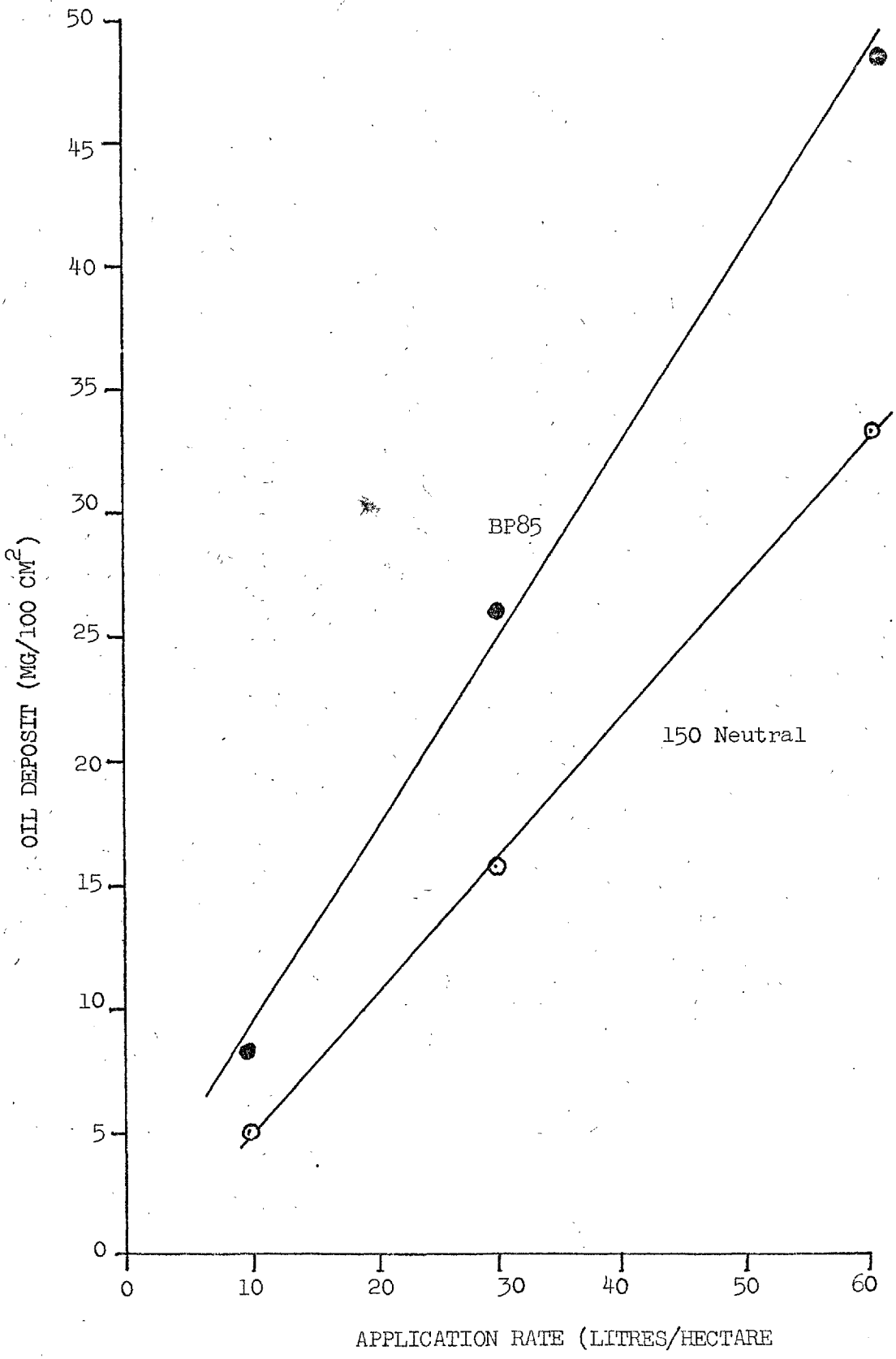


Fig. 47 Calibration of field spray application rate using Minimicron



in Fig. 43. Fractions 13 and 16 volatilized within 24 h and were therefore not included.

D. Oil Deposit

Deposit rates for each oil used were determined by spraying pre-weighed circles of aluminium foil of known area. For BP85 there was no difference in deposit between the side arm and pipette methods described on p.42. Results for the former are shown in Fig. 44. Deposit rates for the (N+P) components of oil fractions also using the side arm method are shown in Fig.45. The (A + H) components were sprayed using the pipette method, results being shown in Fig. 46. Best straight lines have been drawn although statistical analysis has not been carried out. The difference between deposit rates on aluminium foil and cucumber leaves are shown in Table 59 (p.156). Values for vine were not determined.

Commercial deposit rates were determined for vines in France as described on p.47, results for BP85 and 150 Neutral being given in Fig. 47 (results for Gas oil were very erratic and are therefore not included except for the 10 l/ha application rate which gave an average of 4.0 mg/100 cm^2). The actual rates sprayed varied from the desired values (10,30 and 60 l/ha) but have been converted for clarity. Statistical analysis of straight lines has not been carried out.

During work in France it was found that the angle at which the Minimicron was held made a large difference to the amount of oil deposited on either side of the vine leaves. It was held either (1) at knee height and the oil spray directed obliquely at the foliage or (2) as close as possible to the ground with the spray directed upwards into the leaf canopy. Oil deposits were determined using pieces of aluminium foil attached to both upper and lower leaf surfaces, results using BP85 being given in Table 85. The application rate was c.30 l/ha.

Table 85. Variations in oil deposit (mg/100 cm^2) on aluminium foil (attached to upper and lower leaf surfaces) with spraying angle

Spraying angle	Upper surface	Lower surface

Table 85 cont.

(1)	19.2	2.8
(2)	11.5	14.5

APPENDIX II STATISTICAL ANALYSIS

Analyses were carried out on all experiments except with downy mildew of vine where sporulating leaf discs were grouped into sporulation density grades (Tables 10,16 and 17). In most experiments least significant differences (L.S.D's) were calculated by analysis of variance. Where values for disease control and phytotoxicity are given in terms of percentage leaf area affected, L.S.D's were calculated for Horsfall-Barratt grades, since in this grading system, all grades have equal significance whereas percentages do not. Thus whereas mid-points for grades 2 and 3 differ by only 3%, those for grades 6 and 7 differ by 25%.

In experiments on chocolate spot, the same experimental design was used throughout, Last and Hamley (1956), having shown there to be no statistically significant difference between the ability of opposite leaflets of a bifoliate pair to form lesions. Each replicate consisted of one oil sprayed leaflet and one control leaflet. Analyses of differences between controls and treatments were determined using the paired sample 't' test.

Examples of analyses carried out are given below.

1. Germination of sporangia of P.viticola as affected by collection method, collection time and humidity (p.55, Table 5)

Analysis of variance (A.O.V.)

	D.F.	S.S.	M.S.	V.R.
Treatments	11	28708.9	2609.9	420.9***
Error	36	223.5	6.2	
Total	47	28932.4		

Least Significant Differences (L.S.D's) - % germination

3.6 ($P = 0.05$); 4.8 ($P = 0.01$); 6.3 ($P = 0.001$)

Standard Error (S.E.) of treatment means = $\pm$ 1.25%

2. Effect of fraction 22 components and BP85 on vine downy mildew - sporulation grades (p.65, Table 15)

A.O.V.

	D.F.	S.S.	M.S.	V.R.
Treatments	3	26.10	8.70	25.6***
Error	68	23.40	0.34	
Total	71	49.50		

L.S.D. (Grades)

0.38 ($P = 0.05$); 0.51 ($P = 0.01$); 0.66 ($P = 0.001$)

S.E. of treatment means = $\pm$ 0.14

3. Effect of fraction 21 components and BP85 on vine downy mildew - no. of sporangia/disc (p.85, Table 27)

A.O.V.

	D.F.	S.S.	M.S.	V.R.
Treatments	3	789.9	263.3	137.9***
Error	8	15.3	1.9	
Total	11	805.2		

L.S.D. (x 10^{-3} sporangia/disc)

2.55 ($P = P.05$); 3.71 ($P = 0.01$); 5.57 ($P = 0.001$)

S.E. of treatment means = $\pm$ 0.80 x 10^{3} sporangia/disc.

4. Effect of BP85 oil components on cucumber powdery mildew, third arrangement-Horsfall-Barratt grades (p.136, Table 50)

A.O.V.

	D.F.	S.S.	M.S.	V.R.
Treatments	12	137.3	11.44	54.5***
Error	117	24.8	0.21	
Total	129	162.1		

Grade averages

Oil level (ml)	BP85 (W.O.)	BP85 (N + P)	BP85 (O)	BP85 (R)
0.4	9.8	9.8	8.7	10.2
0.8	8.8	8.9	8.4	9.8
1.2	8.3	8.4	8.4	9.0

Control = 12.0

L.S.D.(Grades)

0.4 (P = 0.05); 0.5 (P = 0.01); 0.7 (P = 0.001)

S.E. of treatment means = ± 0.1

5. Comparison of BP85 with gas oil and 150 Neutral for control of cucumber powdery mildew - Horsfall-Barratt grades (p.138, Table 52)

A.O.V.

	D.F.	S.S.	M.S.	V.R.
Treatments	9	665.6	74.0	247***
Error	90	27.4	0.30	
Total	99	693.0		

Grade averages

Oil level (ml)	Gas oil	BP85	150 Neutral
0.4	8.6	9.8	10.6
0.8	6.8	8.8	9.8
1.2	2.0	8.3	9.7

Control = 12.0

L.S.D. (Grades)

0.5 (P = 0.05); 0.7 (P = 0.01); 0.9 (P = 0.01)

S.E. of treatment means = ± 0.2

6. Effect of BP85 on germ tube growth - eye piece units (p. 165, Table 66)

A.O.V.

	D.F.	S.S.	M.S.	V.R.
Treatments	5	27.19	5.44	272***
Error	18	0.37	0.02	
Total	23	27.56		

L.S.D. (eye piece units)

0.2 ($P = 0.05$); 0.3 ($P = 0.01$); 0.4 ($P = 0.001$)

S.E. of treatment means = $\pm$ 0.07

7. Effect of BP85 sprayed at 0, 5, 11, 24 and 36 h after inoculation on hyphal production by Sphaerotheca fuliginea in vivo - % of germinated conidia (p.168, Fig.19)

A.O.V.

	D.F.	S.S.	M.S.	V.R.
Treatments	29	113416	3910.8	144.8***
Error	90	2432	27.0	
Total	119	115848		

L.S.D. (%)

7.3 ($P = 0.05$); 9.6 ($P = 0.01$); 12.4 ($P = 0.001$)

S.E. of treatment means = $\pm$ 2.6%

8. Effect of (N+P) components of oil fractions on chlorophyll content of cucumber leaf discs - D at 665 mμ (p.187, Fig.27)

A.O.V.

	D.F.	S.S.	M.S.	V.R.
Treatments	18	281806.1	15655.9	22.0***
Error	247	175692.2	711.3	
Total	265	457498.3		

L.S.D. ($\underline{D}$ at 665 mμ)

0.020 ($P = 0.05$); 0.026 ($P = 0.01$): 0.034 ($P = 0.001$)

S.E. of treatment means = $\pm$ 0.007

9. Effect of sunlight on the in vitro toxicity of BP85 to Sphaerotheca fuliginea - % germination (p.174)

Treatment means (% germination)

Oil level (ml)	Oil in dark	Oil in sunlight
0.4	41.5	37.7
0.8	29.3	23.5
1.2	22.2	16.5
Control = 56.8		

A.O.V.

	D.F.	S.S.	M.S.	V.R.
Treatments	3	4610.7	1536.9	158.4***
Error	24	232.8	9.7	
Total	27	4843.5		

L.S.D. (%)

4.5 ($P = 0.05$); 6.2 ($P = 0.01$); 8.3 ($P = 0.001$)

S.E. of treatment means = $\pm$ 1.6 %.