PRELIMINARY STUDIES ON SOME DISEASES OF COVER CROPS UNDER RUBBER IN MALAYA.

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Introduction.

The failure of cover crops under rubber may be due to any one of a number of causes. For example the proper inter-changeable nodule bacteria may be absent from the soils. The growth of these organisms may be prevented or inhibited by the presence of some adverse soil condition which results in the production of plants of such lowered vitality that they become more than normally subject to disease organisms which may be present in the soil. Some recent pot culture with *Mimosa invisa* shows that when these plants are grown in a soil taken from a grass blukar* situation on which cattle commonly graze and where the common *Mimosa pudica* is present grows luxuriantly and develops root nodules in abundance. When a part of the same collection of seed was sown in the same soil sterilized or in a hard compact soil on which no vegetation was growing nodules were not produced. The plants were weak and yielded more rapidly to inoculations with *Pythium* and *Rhizoctonia* than the nodulated plants. Cover plants may fail through the absence of certain chemical elements necessary for their normal growth as in the case of alfalfa and clovers which require a certain amount of lime for their best development. The presence of chemical elements in excess of the plant tolerance limits may cause unhealthy conditions or complete failure. The use of varieties poorly adapted to existing soil or climatic conditions are to be considered. Finally the growing of covers without sufficient information with regard to their reaction to light and shade is to be mentioned.

Although failure of cover plants may not always be attributed to the presence of parasitic soil organisms, or of insects or diseases on the aerial parts, the final dissolution of the plants may result from their activities. In some cases parasitic organisms are of first importance. Observations and studies during the past 12 months show that the irregular development of certain cover plants

*Blukar = second growth jungle.

on some soils had to do with definite disease situations separate and apart from any of the other factors mentioned. It is proposed at this time to give a preliminary account of the diseases and causal organisms so far studied and discuss such means of control as may be considered practicable under the varying conditions.

Sclerotium Rolfsii Sacc.

Vigna oligosperma (*Dolichos Hosei*) is frequently observed to die out in more or less circular patches. The patches range in size from a few inches to several feet. Occasionally an entire planting of Vigna on rich humus soil turns brown.

Frequent cultural examination of diseased plants has demonstrated the presence of *Sclerotium Rolfsii* as the causal organism. This fungus is quite common in tropical and sub-tropical regions and frequently causes serious losses of herbaceous garden and field plants. It has been observed on various garden plants throughout Malaya. An examination of the stems and leaves of the affected plants will often reveal a fine cobweb like mycelium upon which are developed small bead-like sclerotia. These bodies resemble small smooth seeds and average about 1 to 2 millimetres in diameter. They are white when young and brown when mature. These sclerotia are richly charged with nutritive material and serve to tide the fungus over periods of drought or other adverse conditions. After periods of dry weather all evidence of fungal mycelia may disappear from the diseased plants but the sclerotia remain and later, on the return of moisture, develop mycelium. Exhaustion of the nutritive substratum will also cause the fungus to develop sclerotia. As rapidly as the cover plant is parasitized the sclerotia appear on the dead parts or if the mycelium on which they develop becomes exhausted through the failure of the vegetative parts of the fungus at the seat of infection, they may sometimes be developed on green parts of the plants. Sclerotial bodies as a rule are potential reserve structures for vegetative propagation. Those found on Vigna and other cover crops have not been observed to germinate in nature but in culture they germinate readily forming a white film of mycelium in abundance on which the sclerotia again develop.

The ability of sclerotia to resist drought is very marked. Sclerotia in cultures brought from America have recently been revived after being dry for over four years. Gadd and Bertus (2,4) report the revival of the sclerotia of *Rhizoctonia solani* after being kept dry for six years. The large sclerotia of certain species of

Poria, Polyporus, Lentinus and *Sparassis* may remain viable in the soil for long periods.

During wet weather the mycelium of *Sclerotium Rolfsii* chiefly grows on the surface of the plants. With a low atmospheric humidity the mycelium is more internal and may be observed on microscopical examination to ramify in the intercellular spaces but may also be intracellular. Not infrequently the affected areas resume growth and may become completely restored which is in the main a reaction to dry weather. The dry weather, especially if the plants are exposed to the sun, checks mycelial development on the serial parts of the plants. The fungus however is not killed. Although it is chiefly active above ground it also develops in the roots underground. This ectrotropic and endotropic habit of the fungus is important from the standpoint of control.

Vigna is very commonly affected. The disease is conspicuous on this plant because of its usual low growing habit and thinness of the cover formed. The fungus has also been found in nature on *Calopogonium mucunoides, Centrosema pubescens and Pueraria javanica.* Inoculation of sterilised soil in seven pots in which the sterilised seeds of *Centrosema plumieri, Mimosa invisa. Crotalaria anagyroides, C. usaramoensis, C. striata, Tephrosia candida* and *Leucaena glauca* (Lamtoro) were sown resulted in a general infection of the seedlings of all seven species. The controls remained free from the disease. The average of the interval between the time of inoculation and the death of the plants was 9 days. The seedlings were attacked as a rule at the surface of the ground and fell over while the tops were still green.

A test to determine the effect of soaking the seeds for 24 hours in water and weak acids on germination and subsequent attack by the fungus resulted in a 25% greater germination and a more rapid germination but the seedlings succumbed to the fungus at an earlier period of growth. This is attributed to the fact that the fungus vegetated more luxuriantly around the seed owing to the soft swollen condition of the seed coat.

Sclerotium Rolfsii has been observed to attack and kill the young hypocotyl of germinating rubber seeds, a condition that has been duplicated by inoculations.

Rhizoctonia solani Kuhn (Corticium vagum B. & C.)

In new plantings where a heavy growth of *Calopogonium* has become established it has been frequently noted that the latter turns

brown in patches and sinks below the general canopy of the cover. These brown patches occur more frequently during rainy weather. On the advent of dry weather the patches ceased to extend and would become obscured by the development of the surrounding plants. An examination of the stems in contact with the soil and of the roots showed that a fungus had penetrated and was apparently the cause of the death of the cortex. The parenchymatic tissues of the woody parts in microscopical preparations were found to be completely destroyed leaving the ligneous portions of the stem quite disassociated.

Growing over the affected parts of the plants above ground a fine fungous web was discernible. This mycelial web was made up of colourless yellowish brown septate hyphae averaging in diameter 7.5—12 microns. These hyphae frequently caused the leaves and stems to adhere. The general debris on the ground matted together by the hyphae formed a whitish layer. This layer in the main consisted of the same type of hyphae as those found on the aerial parts of the plants. On the green leaves and stems the web assumed a more flocculent and powdery appearance and showed on examination to consist of separate hyphae 6—12 microns in diameter with free standing basidia bearing usually four hyaline, oval, apiculate spores averaging 6.5—12 x 4—5.5 microns. On the diseased leaves and stems near the soil flocculent rounded hyphal masses were relatively abundant. These masses very readily formed sclerotia when the affected parts were held in a damp chamber. The sclerotia varied in size from a half to 4 or 5 millimetres and frequently coalesced to form larger structures. They were rounded or flattened and varied in colour from white to a deep chestnut or purplish brown. Under the lens the surface appeared smooth or irregularly pitted and covered with a hyphal investment, depending upon age and degree of moisture. The mature sclerotia were usually covered with droplets of a brown liquid during wet weather. Their structure was homogeneous being composed of fairly uniform cells without differentiation. The foregoing describes the main characters of the fungus as determined from study of field material.

The fungus was readily brought into pure culture from the hypha from the sclerotial masses and from the basidial spores. Each of these structures in culture produced the complete life history of the fungus. The study of cultures and subsequent inoculation work shows quite clearly that the fungus is identical with *Rhizoctonia solani*. It agrees in most particulars with authentic cultures from America and in the main essentials with a culture under the above name kindly sent to the writer by Mr. Murray of the Rubber Research Scheme in Ceylon. The Corticium stage

which has been surprisingly easy to produce both from field material in the laboratory and in pure culture agrees with authentic material in the writer's herbarium. The spores in the type material are slightly smaller and more conspicuously apiculate than in most British, American and African specimens.

Since the original collection on *Calopogonium* the fungus has been found causing brown patch effects in densely growing *Vigna*. These are the only two cover crop plants so far found infected by the writer under field conditions in Malaya. On the other hand it has been isolated from diseased lettuce. The fungus is widely distributed in temperate regions and the following brief review of some recent literature shows it to be common in tropical countries.

The "Mopo" disease of the Cinchona seed-beds in Java as originally reported by Moens and more definitely studied by Rant is referred to *Rhizoctonia solani* by Duggar (3), Gandrup (5) more recently reports the fungus in Java on *Vigna*. The writer obtained cultures of a fungus from diseased patches of *Vigna* and from a garden plant in Sumatra which later developed all the characteristics of *Rhizoctonia solani*. Gadd and Bertus (4), give a full description of the fungus and the disease on Vigna, paddy, groundnut, plantain and acacia in Ceylon. Shaw (12) reports the fungus in India. The fungus has been reported from South America and the West Indies. Reinking (8) describes a *Rhizoctonia* as causing a damping off of Citrus seedlings in the Philippines which appears to be the species under discussion. Schweizer (10) reports a single collection of a fungus on leaves and branches of closely planted Hevea in Java which he thinks is probably identical with or is a physiological form of *Rhizoctonia solani*. The fungus is strongly parasitic on *Vigna* but the local Rhizoctonia on *Vigna* did not grow well on Hevea. The writer's inoculation experiments with *R. solani* from *Vigna* show that it will parasitise young 5 days old Hevea seedlings causing a collar rot. Bertus (1) using a strain of *R. solani* causing a die-back of tea seedlings failed to infect seedlings of French beans, cotton, cowpea, groundnut and Lima bean. Similarly a strain from *Vigna* would not infect tea seedlings at the ground level. All this indicates considerable variation in the pathogenicity of *R. solani* from certain hosts when brought in contact with its usual hosts but as Bertus suggests it may represent a condition wherein the proper environment for infection was not provided.

The pathogenicity of the fungus from *Vigna* and *Calopogonium* has been fully established by inoculations from pure cultures on seedlings of *Vigna oligosperma*, *Calopogonium mucunoides*,

Tephrosia candida, *Mimosa invisa* and *Crotalaria striata* grown from sterilized seed in sterilized soil. Depending upon their age the seedlings either "damped off" at the ground level or were ringed at that point with the temporary formation of a callus just above the zone of infection. The latter remained alive for a short time but eventually succumbed to the fungus and remained standing. The early applications of a fungicide occasionally arrested the action of the fungus with the result that new roots were developed from the callus above and the plants survived. Covering the seedlings with a white damp cloth or with celluloid cylinders usually resulted in the fungus spreading over the stems and leaves of the plants after the manner of the fungus in nature.

No real distinctions in general behaviour or appearance have been noted between the culture obtained from *Vigna* and *Calopogonium* in Malaya, *Vigna* in Sumatra and the culture from *Vigna* in Ceylon. Evidently a single strain of the fungus is involved. Sowing of infected soil with sterilised seed of one species of cover in which another had succumbed to the disease with no marked difference in the behaviour of the fungus indicates the uniform character of the parasites from the different regions and substrata. Infections of cuttings of *Calopogonium* and *Vigna* were not successful until after the plants had formed a dense canopy over the tops of the pots simulating field conditions.

Pythium sp.

Centrosema pubescens on two widely separated estates previously cultivated has been found to be infected with a species of *Pythium*. The diseased plants grew on a soil rich in humus under shade and in a damp situation. The affected plants appeared in small isolated patches and were characterized by a lighter browning of the leaves than is usual with such infections. A very fine white web-like mycelium over-ran the plants causing the leaves to adhere and to remain suspended if detached from the stems. The older stems usually remained living, only the growing ends being parasitized. The mycelium also grew over the soil and the dead plant material beneath. A small quantity of the soil and dead and living leaves were introduced among young *Centrosema* plants in pots. Within ten days the plants were infected and were killed. Pure cultures were then made and tested on seedlings growing in sterilized soil. Out of fifty seedlings so treated 13 were infected at the ground level and died. The fungus was then tested on seedlings of *Indigofera endecaphylla* and *Vigna* with a like result.

The fungus appears to affect first the roots, especially the fine ones, then grows up around the stem and if the cover is dense the mycelium spreads over the stems and leaves. The infected rootlets when planted out yielded Pythium in all cases save one which produced a *Rhizoctonia* not previously mentioned in this paper. Infection is apparently greatly increased by excessive soil moisture. When infected plants in pots were kept well watered they damped off more rapidly than when dry. This agrees with the behaviour of the fungus in the open.

Further studies are being made of the causal organism and to determine the extent of the damage it may cause to cover crops. Its taxonomy is indefinite but its conidia and oospores seem to agree with the debaryanum type and with a form found on germinating rubber seeds. Sharples (11) and Thompson (13) report the isolation of a *Pythium* from Patch Canker and from late maturing rubber pods but it is stated that the fungus has not recently been noticed.

Control.

The measures that may be adopted to retard or control the three soil inhabiting fungi described above meets with difficulty in practice under field conditions. The measures available may be discussed under the headings of isolation, nurseries, soil sterilization, selection of site, combination planting, clean seed, rotation and immunity.

Isolation.—The first noticeable effect of the action of soil fungi as previously stated is the dying out of the cover in small patches. This may take place on any type of soil under wet weather conditions but is most noticeable on moist soil of high humus content.

As a first step when patch infections are noticed the patches may be isolated by a well defined trench. The fungi in the case of the herbaceous type of cover do not penetrate the soil to any great depth hence the trench need not be more than a foot and a half deep. If nothing more is done the trench should be kept free from the vines otherwise the fungus may cross to the other side. The trench should enclose a zone of healthy plants. A second step is to disinfect thoroughly the isolated patches with any of the soil fungicides named below. The infected plants should be removed from the area and burnt. Gadd & Bertus (4) in Ceylon in the case of *Rhizoctonia solani* recommended that the diseased plants should be collected in tins for removal in order to avoid dropping sclerotia on clean soil. They further recommend

that disease areas should be planted to *Indigofera endecaphylla* or *Centrosema pubescens* which they found during cultural experiments to be immune. On the other hand Schweizer (9) finds that the former is attacked by *Rhizoctonia solani* in Java. Gandrup (5) also in Java reports the immunity of *Centrosema* and recommends its use. The writer's experiments are not yet conclusive on this point and will be reported later. *Centrosema pubescens* has not been found infected in the field.

The difficulty sometimes experienced in establishing cover crops even on soils favourable to their development is due in part to faulty germination, damping off of the seedlings by soil fungi, failure of the seedlings to grow vigorously from the beginning and hence inability to withstand inhospitable conditions then present or which may appear later. These conditions may be in a large measure counteracted by establishing on soil free from infection, sizable nurseries from which vigorous seedlings or cuttings may be taken at any time. In view of the fact that the fungi described attack mature and vigorously growing plants with difficulty under ordinary conditions and are largely active during the wettest parts of the year, it is desirable that the planting on the permanent site be started off with well grown plants.

Soil Sterilization.—Most tropical soils contain a number of pathogenic fungi. The "damping off" of cover crop seedlings is a good example of their activities. In a small cover crop nursery this trouble can be prevented by soil sterilization. The two methods usually employed to sterilize soil are either to heat the soil directly or to use disinfectants. It appears desirable to describe these methods since they may be employed by the rubber planter for various purposes, for example to increase the percentage of germination of rubber seeds when planted in nurseries.

By heat.—Boiling water may be copiously poured or sprayed over the beds from an oil tin with fairly large perforations in the bottom. This will destroy most soil fungi. The top soil may be shovelled on to sheet or roofing iron elevated on iron posts about two feet from the ground under which a fire is built. The soil should be heated thoroughly for about one hour at a temperature just high enough so that the soil may not be picked up in the hand, say 70° to 100° C. The humus or organic material in the soil should not be burned. To make the control more efficient both the soil of the beds and the soil that has been heated may be sprayed with a two per cent formalin solution. The heated soil may then be returned to the beds. After two or three days the soil will be sufficiently serated for sowing the seeds.

The heating of the soil by the burning of new clearings is undoubtedly a factor in freeing the upper soil layer of many pathogenic fungi and reduces the incidence of disease appreciably. Covers established on soil that has been very thoroughly heated due to the burning of piled debris after the first broadcast burning are observed as a rule to be very luxurious and free from disease. *Wood ashes and air-slaked lime are good disinfectants* in the case of some soil fungi which work only in an acid soil and may be used to neutralise the after-effects of acid treatment. Duggar states that cultural studies have shown that *R. solani* is able to withstand a high percentage of alkalinity but believes the application of lime may restrain the activity of the fungus indirectly by raising the resistance of the host.

By disinfectants.—Patch infections of *Rhizoctonia solani* and *Sclerotium Rolfsii* under old rubber have been prevented from spreading by the direct application of one percent solution of copper sulphate. It may also be applied directly to the nursery beds. Experiments with pot cultures in which there was a high mortality among seedlings after artificially introducing *Rhizoctonia, Sclerotium* and *Pythium* proved that copper sulphate would completely prevent further damping off. Hartley's (6) extensive work on damping off of coniferous seedlings demonstrated the efficiency of commercial sulphuric acid (1-2 oz. per gallon of water at the rate of 1 quart per square foot before and after seeding) when applied to the seed-bed soil but it appears that some cover crops are not especially tolerant of acid treatment. The treatment however may hasten seed germination. It is well to state acid should be dissolved by pouring it into the water—never by the reverse process. Wooden or earthenware containers should be used for acid and copper sulphate and should be applied with sprinklers which have been coated inside with hot paraffin wax.

The formalin treatment of seed-bed soil is more efficient if the soil is afterwards covered with tarpaulin or sacking to prevent loss of the disinfectant by evaporation. Some three days after the removal of the covering the seed may be sown. Clean sand or coarsely powered charcoal spread on soil after seeding may be of value in counteracting fungi but these are amendments rather than disinfectants. If a strong solution of formalin is used it should be applied at least one week before seeding.

Experiments on seedlings in pots and on various garden flowers growing in beds infected with *Rhizoctonia, Pythium* and *Sclerotium* have demonstrated the efficiency of a standard normal solution of Semesan ($\frac{1}{3}$ oz. to 1 gall. of water, 0.25 per cent.) and

of Uspulun (1 oz. to 3 galls. water) in controlling damping off. Information concerning the nature, source and use of these fungicides may be had on application.

Selection of Site.—In field practice under shade where it is proposed to establish *Vigna* it is desirable to select the most favourable sites, viz. soil that is fertile, well drained and with an average supply of soil moisture. Planting may then be done during the dry periods of the year with greater assurance of success. The atmospheric moisture at the ground level which greatly influences the development of soil fungi will be less and the seedlings or cuttings will come away with greater freedom from infection. These areas may be carefully inspected, all infected patches, if any, eliminated and the areas used as a source of supply for other parts of the estate. When planting rooted plants or cuttings over-crowding should be avoided. Field observations and pot culture work demonstrate that the moisture factor is of great importance in relation to severity of attack. Park (7) especially calls attention to this fact by some recent work with *Rhizoctonia solani* on cotton seedlings.

The mortality among seedlings of cover crops especially on damp and poorly aerated soils may be expected to be high. A close examination of seedlings resulting from any of the practical methods of planting whether in drills, manured or unmanured, broadcasted, soaked or unsoaked always shows that a considerable number of the young plants have succumbed after a few days growth to parasitic fungi in the soil. Since the seedlings in the young stage are very liable to attack it is desirable to judge well the nature of the soil as regards drainage and soil moisture and as an alternative in some cases establish the cover by the use of cuttings or basket plants. The firm tissues of the latter at the surface of the ground where the fungus attack usually begins, prevents or retards infection until the cover is established.

Combination Planting:—*Calopogonium*, *Centrosema*, and *Vigna* when sown together may intensify infection on the most susceptible species but if the plants can be brought through the more susceptible early periods of growth it may be expected that the natural succession of the plants with increasing shade will work out successfully.

Clean seed:—In the case of the fungi concerned infected seed is unlikely if they are properly collected. Seed collected from heavily infected plants may contain sclerotia. Floating the seed of several species in water resulted in the discovery of a single

sclerotium. This, when tested in culture, proved to be *Sclerotium Rolfsii*. Soaking seed in acid or other liquids to promote rapid germination may not be desirable except for particular soil types. Seed disinfection may be practised where seed has been taken from diseased plants.

Rotation:—If it can be proven that certain species of cover plants are not susceptible to the soil fungi described in this paper crop rotation would be of considerable use. Soil fungi of the sclerotial type may survive in the absence of host plants for long periods of time. If susceptible plants are continuously grown the soil becomes infected and this results in greater damage during the wet months. The fact that cover plants are being extensively employed following a long period of clean weeding which tended to prevent the development of an infected soil makes it desirable to sustain the present favourable situation as much as possible by growing resistant species.

Immunity:—On those soils where cover crops have failed to develop owing to the presence of disease an intensive study of the diseased plants and conditions encountered is desirable. It is hoped that natural immunity may be fully established under field conditions for some of the best covers now in use in Malaya as has been indicated above. The cover crops used in different tropical countries are being assembled. It is possible that the variety and legume crop trials on diseased soil now being instituted may result in the discovery of other plants or varieties of plants now grown, which would not be affected by the factors enumerated.

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