

THE EFFECT OF GROWING PEPPERMINT ON THE PERSISTENCE  
OF CLUBROOT OF CABBAGE IN MUCK SOIL

By

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## INTRODUCTION

Plant associations that produce beneficial or ill effects on one another have long been recognized. Crop rotations are considered essential in most cropping systems, yet there is little understanding of how one crop influences the subsequent development of another.

The work herein discussed, results in part from an observation made in 1945 on a field known to have been contaminated previously with Plasmodiophora brassicae, Wor., the organism that causes clubroot of cabbage. Gries (13), Pryor (30), and Walker and Hooker (46) reported on the effect of potassium on increasing or decreasing the incidence of clubroot. These findings prompted the author to establish field plots, at the Northern Indiana Muck Crops Experimental Farm, to test their practicability. Contrary to expectation, the cabbage grew well on all plots, and no clubs were found on any plants. Reference was made to the field history and it was found that peppermint (Mentha piperita Linn.) had been grown on this field for three years preceding the cabbage crop. A field of cabbage only thirty feet away, on soil not previously planted to mint, was destroyed completely by clubroot. Both cabbage, which is commercially important and always jeopardized by clubroot, and peppermint, which is of major importance in Indiana and Michigan, were therefore involved. Both crops

are grown in the same parts of the states of Michigan and Indiana, but not necessarily in the same locality and on the same soil types. Should mint\* in rotation with cabbage prove beneficial to cabbage production in the areas threatened with extinction because of the clubroot, the mint crop could be introduced and grown either as a cash crop or as a green manure crop for the control of the clubroot disease.

This problem involves further, the recognized fact among growers and those familiar with research dealing with peppermint, that the yield of peppermint oil declines after several years of continuous mint cropping on organic soils. No program of fertilization has been found that will restore the original vigor and yield to the planting. A common physiological disease termed "squirrel ears" by growers, often develops in these fields. Affected plants seem to be prematurely old physiologically. They become "hard" and the yield is depressed.

Other crops are known to be depressed when grown following mint. Experienced farmers, for example, will not plant onions in a field that has been in mint and some growers have reported that potato scab is more serious following this crop. Corn, however, exhibits no ill effects following a peppermint crop.

The objectives of this work were to study some practical

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\*The word "mint" will be used interchangeably with peppermint.

controls of clubroot, with special attention to the effect of peppermint on the persistence of the organism, and to determine, if possible, the mechanism of its control.

## REVIEW OF LITERATURE

No direct reference to the association of the peppermint plant to cabbage or the clubroot organism has been found.

The literature cited, therefore, must pertain to the clubroot organism and its control, to the various types of plant associations dealing with control of plant diseases, and to references to the mint plant and products produced by it. Because of the abstractness of the problem, literature bearing on the subject, even in a remote way has been included here to present more effectively the background.

Michael Stephanovitch Woronin (56) first described the causal organism that produced clubroot of cabbage and named it Plasmodiophora brassicae. The disease was not new but the cabbage crop was of such high value to the Russian market gardener that the Royal Russian Gardening Society of St. Petersburg posted a prize for a scientific solution to the problem. Independent of this stimulus, Woronin completed his work in 1878. The study is especially memorable because little has been added to the essential facts in the past 73 years.

Woronin stated that the disease was known by many names. It was called Kapoustanaja (crucifer hernia) in Russia; Kohlhernie and Kropf des Kohles in Germany; and

Vingerziekte\* or Malade digetoire in Belgium. In Spain, Ruiz Diaz de Isla (18) attributed the disease to a syphilitic infection, and in England it was called clubbing, clubroot, ambury, hanbury, and fingers and toes.

Woronin described the swellings on cabbage roots and mentioned that the largest ones were usually found on the tap-root. Clubs exist for fairly long periods of time in dry soil but in wet soil they rot rapidly. The parenchymatous tissue falls away from the vascular tissues, which remain for some time. Usually the decay begins at the deepest point in the soil. Auxiliary roots are sometimes produced from leaf scars on the stem when the root is diseased. Woronin pointed out that the disease attacks most all cultivated crucifers such as cabbage, turnips, and rape, and most of the weed members of the crucifer family.

In 1878, Woronin stated "In my opinion, the absolute eradication of hernia on the cabbage plant is impossible. Anything that would destroy the organism would destroy the plant." He made three recommendations for the control of this disease:

1. All stems and roots should be gathered and burned.
2. Sort plants before setting in field.
3. Practice a rational crop rotation.

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\*According to Karling (18) the word Vingerziekte mentioned by Woronin and others is unknown in Belgium.



He then stated that the last measure did not work, because perhaps not enough care was taken in its practice.

Karling (18) published a treatise entitled "The Plasmodiophorales", in which he cited 586 different references on the clubroot organism and its control. He stated that there are probably additional references that were omitted accidentally. This work reviewed the literature up to 1942. Except for papers of special significance, therefore, reference to papers prior to 1941 will be listed as taken from Karling (18).

A description of the causal organism follows (18):

Resting spores globose, spherical 1.6-4.3 $\mu$ , average 3.9 $\mu$ , oval, ellipsoidal, 4.6x6 $\mu$ , sometimes constricted, elongate and irregular, 2.5-6.9 $\mu$  with smooth, relatively thin, hyaline walls. Zoospores pyriform, spherical, 2.5-3.5 $\mu$ , swimming rapidly and becoming intermittently amoeboid. Sporangial plasmodia variable in size. Zoosporangia few or numerous, small, oval, spherical, 6.0-6.5 $\mu$ , angular and elongate with thin, hyaline walls; producing 4 to 8 zoospores which are liberated by the collapse of the sporangium wall. Sporogenous plasmodia 100-200 $\mu$  in diameter, hyaline to pale-grey in color, amoeboid; encysting occasionally, undergoing schizogony into uni- and multinucleate meronts.

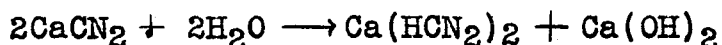
Ayers (1) studied the life history of the clubroot organism and observed that the resting spores germinate to give rise to biflagellate zoospores. When a zoospore comes in contact with a root hair or the epidermal wall of a cruciferous plant, it settles down as an amoeba and

penetrates the wall to form a young thallus within the host cell. The thallus may or may not become enlarged before the protoplasm undergoes change and an irregular cluster of zoosporangia is formed. Each zoosporangium contains 4 to 8 zoospores, which at maturity are discharged from the root hair if free moisture is present, otherwise the fungal protoplasm disintegrates. Only 4 to 6 days are required from the time of infection to the discharge of the zoospores from the zoosporangium. The part that these half-sized zoospores play in the life history of the disease is not known, but it is important to point out that studies on the control of the disease have not taken them into consideration. Heating (18) the soil to 140° F for one-half hour kills the spores. Many chemicals have been used with varying results. Some capable of killing the organism will also kill the host or depress the yield of the crop. No practical field control was reported from chemical treatment although most of the standard fungicides such as mercuric chloride, formalin, and sulphur were used as well as many other chemicals. More recently, Walker (49) working with solutions to be applied at transplanting time has pointed out that mercuric chloride was the only material giving good results. This would require about three pounds of mercuric chloride in 350 gallons of water per acre. Green (11,12) observed good results with the use of a calomel dust applied in the row

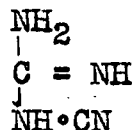
before planting.

Application of lime (18) has been the most widely recommended practice in the field, yet the results have been variable. Over 50 workers reported beneficial effects from lime, while about half that number have reported unfavorable results. The amounts recommended vary from less than one ton up to sixteen tons of lime per acre. The form of the lime is reported to be important but ground limestone, hydrate of lime, slaked lime, calcium cyanamide ( $\text{CaCN}_2$ ) and calcium chloride have been used by various workers with contradictory results. It was believed earlier that lime tends to retard spore germination but it has since been shown that spores germinate over a wide range of pH. Since inhibition by the salts of other metals has been shown, some workers have concluded that the essential factor is the presence of free hydroxyl ions in a soil. Karling (18) reported that Bremer, working with portions of infected cabbage roots buried in different soils, concluded that the spores would not germinate as rapidly in alkaline soils as in acid soils and, therefore, the organism would remain in alkaline soils for a greater length of time. He used a technique for extracting the spores from the soil first by plasmolysis in salt solution and then by deplasmolysis in water. No other reference to this approach has been found. Dillon-Weston (7), Daines (6), Garrett (9), Chupp (3) and many other writers have continued to recommend the application of lime even though its use has

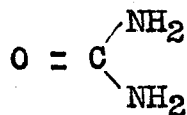
not been particularly effective. Walker (45) states that if the pH of the soil is maintained above 7.2 along with sufficient soil moisture, the effects of the disease will not be severe. He attributes the failure of liming under certain conditions to the fact that plant roots give off  $\text{CO}_2$ , forming more carbonic acid in the soil close to the plant roots than can be neutralized by the lime, thus allowing the organism to attack the roots. Walker (47) found that Calcium Cyanamid gave erratic results in relation to control of the disease. According to Crowther and Richardson (5) calcium cyanamide breaks down into different decomposition products, depending on the soil reaction. In a water solution, free cyanamide is produced,



The free cyanamide in an alkaline solution polymerizes to form dicyanodiamide,



In an acid solution, the free cyanamide hydrolyzes to form urea,



which is ineffective in killing the spores of the clubroot organism. The most effective control with Cyanamid has been observed when the soil was already approaching a neutral reaction before the application of Calcium Cyanamid was made.

Observations on the basic fertilizing elements offer

no practical solution to the problem, although a deficiency of potassium decreases the disease as shown by Pryor (30), Gries (13) and Walker (43). A deficiency of potassium would also decrease the yield of cabbage on organic soils below economical commercial production.

Soil drainage (18) was early considered as a method of control but it has been shown that clubroot flourishes on dry soil as well as on wet and that other factors probably entered into control by drainage. Sandy soils, clay soils and soils with abundant humus have all been reported as favoring the disease and Walker (46) produced the disease successfully in quartz sand and nutrient solution. Soils (18) that warm up early in the spring are supposed to be more conducive to fostering the infection but temperature has been shown to have little effect on the organism. It must be, therefore, the lower number of spores required for infection in the light soil rather than a temperature relation affecting these soils. According to Karling (18), Hayunga and Jonson and Bremer found the disease to be less severe on marsh or heath soils; in line with this, Fedotova claimed that soils with a low organic matter content required less concentration of spores to cause disease than soils with a high organic matter content.

Several other approaches to control of Plasmodiophora brassicae Wor. have been studied. Sanitary practices are

recommended by many workers (18) particularly in relation to the use of manure that is contaminated with the organism. It is known that the organism can pass through the digestive system of an animal without being destroyed. Burning of infected roots and burial to a depth of 80 cms have been recommended. Plowing under to a depth of 20 to 30 cms is not effective because it has been reported that infection can take place to a depth of 30 cms. Wilson (55) stated that in muck soils in Ohio the clubroot organism was found to a depth of 12 inches. Potter (29) stated that the organism lives only in the top 5 to 6 inches in mineral soil.

Plant beds (18) must be disease free and transplants should be inspected before using. Disinfection of the seed, seedbed and soil have all been investigated. Warne (50) has shown that clubroot can be carried on the seed coat of Brassica species and suggests disinfection of the seed as a control measure.

Various degrees of resistance to clubroot have been reported (18) among the cultivated and wild crucifers. None seem to be one-hundred percent immune and many that are resistant under one set of conditions or in one location, are not resistant in other locations and under different environmental conditions. Karling (18), listed at least 318 species in 59 genera of the Cruciferaceae that had been examined for clubroot. Among these, all but 89 species and 8 genera were reported to be infected if

clubroot was present. The small numbers of plants investigated may account for lack of clubroot on some of these.

Host plants of P. brassicae seem to be limited to members of the Cruciferaceae and all reports of clubbing on species outside this family have been proven false. The nature of resistance or susceptibility is important since it bears on control. It has been suggested that biological strains of the organism differ in degree of virulence (45). This might account for the different degrees of resistance or susceptibility reported by various research workers.

Ssacharoff (18) in 1916, believed resistance was correlated with low sugar content in the cell sap, but Whitehead (18) later claimed that there was no association between sugar content of the roots and susceptibility to the organism.

Rochlin (31) in 1933 published her work dealing with the nature of resistance to clubroot. She concluded that no resistance could be attributed to differences in anatomical structure of the roots in young plants. In adult plants, the disease is restricted or hindered by layers of cork, collenchyma, or by compact woody tissue. She further attributed resistance to the amount of glucosides contained in a plant which, on hydrolysis with myrosin, produced highly pungent mustard oils. Since that time, Stahmann et al. (36) and Hooker, Walker and Link (16) have shown that inhibition or inactivation of P. brassicae spores is produced by mustard oils, but that the exudation of

these oils by the roots of crucifers does not take place. Smith, Link and Walker (33) showed also that resistance to clubroot was not due in any large part to the presence of pre-formed extractable phenolic or acidic fungicidal constituents in the cortical tissues of the fleshy roots of crucifers.

During the last decade, Walker (44) and Walker and Larson (48) have reported progress in breeding for clubroot resistance. From kale-cabbage hybrid rogues found in a Wisconsin cabbage field in 1941, resistant progenies have been developed. The  $f_1$  of crosses between cabbage and selfed progenies of the kale-cabbage rogues segregated heavily to susceptible plants while in the  $f_2$  a small number of resistant individuals appeared. These were divided equally in type as follows: kale type, intermediate type, and heading type. From this last group selections were made toward a commercial type of cabbage. Walker (45) states that the garden kales are susceptible but that certain varieties of stock kale are resistant.

There remains another possible approach to clubroot control -- that of the association of plants in rotation. This phase has been observed and studied by more than 20 workers (18). It has been shown (9) that spores of P. brassicae can live in soil without the presence of a host plant for 7 to 8 years. Obviously then, a long rotation period without a susceptible host plant would be essential for control by this method unless the association



of another plant affected the viability of the organism. Halstead (14) reported a five-fold increase in turnips on land which had previously been planted to buckwheat. The buckwheat plants were chopped up and worked into the soil. Less clubbing developed on the first crop but the beneficial results were not evident the second year. Murphy (18) recommended that cabbage be alternated with carrots. Müller-Thurgau and Osterwalder and Blunck (18) claimed that three years of beans were effective in controlling the clubroot organism in the soil. The species of beans was not given. Pettera (18), in 1917, maintained that Physostegia virginica Linn., a member of the family Labiatae, bachelors button, Achillea ptarmica, of the family Compositae, goatsbeard or astilbe of the family Saxifragaceae, and pyrethrum of the family Compositae, all have an inhibitory effect on the clubroot organism and inactivate the spores within three years. Fedorintschik (8) recommended that grass and clover should be used during the last two years of a rotation to avoid plowing the land.

Examples of other crops that have beneficial effects on the control of soil-borne diseases are cited by Smith (34), by Atkinson and Rouatt as reported by Lockhead (22), who show that residues from soybeans decrease the incidence of potato scab. Hildebrand and West (15) incorporated plant remains of soybean, corn, red clover, and

timothy into soil in which strawberry plants were grown. Untreated soil, sterilized soil, and soil treated with manure were also used. The plants in the sterilized soil and those in the soybean series remained free of the root rot. West and Hildebrand (54) again showed that soybean plant material reduced the incidence of root rot on strawberry but that red clover did not and warned that soybeans do not inhibit all root rot diseases, pointing out that Beckley and Koch had accentuated root rot of tobacco by preceding the tobacco crop with soybeans. Hopkins (17) discusses the resistance of the American grape root stocks to the vine phylloxera. This soil-borne aphid attacks the native French root stock but is starved out of the soil when the French vine is grafted on American roots.

Besides the elimination of the host plant, which is considered to be the essence of a crop rotation as far as the clubroot organism might be concerned, two other possible effects of plant associations are suggested in the literature. Although not dealing with the peppermint and cabbage plants, these references refer to type situations which could apply to this problem. These effects may be grouped as, first a change in the microflora of the soil such that dominant organisms compete with and subdue the disease organisms by sheer vigor, and, secondly the effect of exudates from roots of plants that may be toxic or antibiotic to soil-borne organisms directly, or toxic, antibiotic, or stimulating to organisms which in turn may

be toxic or antibiotic on other soil inhabiting organisms.

The effect of plants on the soil microflora has been studied by Starkey (37). He grew mangel beets, barley, maize, rape, vetch, and soybean. With his technique, he observed ~~no~~ striking differences between the different plant residues but he did observe an increase in the number of organisms in the rhizosphere as the crops matured. Lockhead, Timonin and West (23) found that varieties of tobacco and flax which were resistant to root rot supported a lower population of bacteria, actinomycetes, and fungi in the rhizosphere of the plants susceptible to the disease. In 1946, Sanford (32) pointed out again the beneficial effect of crop sequence or summer fallow on reducing soil-borne diseases but admitted that the mechanism of that beneficial effect was still obscure. Garrett (10) in illustrating the effect of a strong organism over a less vigorous one, cited the work of Millard and Taylor in which Actinomyces scabies (now Streptomyces scabies) is killed by inoculating the soil medium with Actinomyces precox which is also an obligate parasite but more vigorous than S. scabies. He also referred to the work of King who used organic manures to build up the population of an organism which in turn attacks Phymatotrichum omnivorum, the organism that causes cotton root rot. Garrett has defined two types of soil fungi, soil inhabitants and soil invaders.

Soil inhabitants are considered to be primitive or unspecialized parasites with a wide host range; these fungi are distributed throughout the soil, and their parasitism appears to be incidental to their saprophytic existence as members of the general soil microflora.

Soil invaders, to which class the majority of the root infecting fungi seem to belong, are more highly specialized parasites, (the group to which P. brassicae belongs) the presence of soil fungi in the soil is generally closely associated with that of their host plants. In the continued absence of the host plant, such fungi die out in the soil, owing to their inability to compete with the soil saprophytes for an existence on non-living organic matter. This close association between soil invaders and their host plants thus seems to be enforced by the competition of the general soil microflora.

Weindling (52) emphasized that the term "microbial antagonisms" now referred to complexes as well as to any two organisms. He pointed out that the mechanism of microbial antagonisms is very complex and that there are synergistic, competitive, parasitic and toxic activities of one group toward another. It is now recognized that it is practically impossible to evaluate the relative importance of single factors. He discussed two approaches to improving on nature's biological control -- the inoculation of the soil with specific microorganisms, which procedure has not given striking results except in legume inoculation, and secondly, providing more favorable conditions for organisms in the soil. As examples of the latter he mentioned the control of cotton and cereal root

rots by applications of organic manures, the partial control of potato scab by green manuring and the control of rhizoctonia, causing damping off, by acidifying the surface soil. These indirect approaches, such as the modification of the soil, have been most successful.

Russian workers have advocated direct treatment with cultured microorganisms, but so far other treatments have been simpler, cheaper and more effective.

Lockhead (21) and Katznelson, Lockhead and Timonin (19) have pointed out how the growing plant influences the balance between groups of soil organisms. Katznelson and others (19) list the various modifications of the soil which plants can provoke as follows: (a) lowering the concentrations of certain mineral nutrients in the soil due to their absorption, (b) partial desiccation of the soil by absorption of water, (c) increase in soil carbonates following root excretion of carbon dioxide, (d) contribution of microbial foods by sloughed off root portions or excretions. Emphasizing the rhizosphere effect, they point out that the increased density of organisms in the root zone with the expected associative and antagonistic relationships will result in pronounced favorable or unfavorable effects on the plant. They introduce the R:S ratio (rhizosphere to soil at a distance from the root) and point out that for evaluating the influence of soil type, treatments, and other factors on the root surface microflora, this ratio is of fundamental importance.

In general, age and nature of the plant affect the number and type of organisms. Very little work has been done on the effects of plants on fungi. It was reported that ~~isolations from cork roots~~ on acid soils yielded *Trichoderma* cultures predominantly whereas in alkaline soils the roots seemed to be associated principally with species of *Penicillia*. Waksman (41) also indicated the antagonistic effects of *Trichoderma lignorum* which, when applied to seed infested with *Helminthosporium sativum*, inhibited this organism. The rhizosphere of resistant varieties of a crop supports a different soil population from that of a susceptible variety. Timonin (39) noted higher numbers of bacteria and, to a lesser extent, of fungi in the rhizosphere of varieties of flax and tobacco susceptible to soil-borne plant pathogens, though these plants were free from disease. It was suggested that these results reflected inherent differences in physiological function of the plants, making conditions generally more favorable for the growth of the organism around the susceptible plant.

West and Lockhead (53) reported that thiamine, biotin, and certain amino acids were excreted from the roots of plants and that these substances favored the development of those types of microorganisms that had complex nutritive requirements. Waksman (42) outlined the interrelations of microorganisms in natural environments. He

classifies them as follows: (a) favorable effects, such as the production of a growth substance by one organism to promote the growth of another (b) strict symbiosis, which benefits both, and (c) unfavorable effects such as

- (1) production of an acid by one to destroy another
- (2) nutrient competition
- (3) antagonism or antibiosis

Sobels (35) demonstrated the production of antibiotics by myxomycetes and Lockhead and Landerkin (22) have diagrammed the interrelation and antagonistic effects of 11 actinomycetes on the organism causing potato scab, Streptomyces scabies. All strains of actinomycetes tested were antagonistic to Streptomyces scabies, but in turn most of the 11 strains of actinomycetes were antagonistic to each other. Lockhead and Landerkin state "There is increasing evidence of microbiological equilibrium in soil, though one ever changing under the influence of temperature, moisture, soil treatment and cropping systems...the microbiological balance is affected by the antagonistic effects exerted on certain microorganisms by others". Brian (2) in June, 1949, concluded that the capacity to produce antibiotic substances is common among all groups of soil microorganisms. Evidence was presented to show that there is reason to believe that production of antibiotics is responsible (in part) for the numerous cases of antagonistic relationships between soil microorganisms. He also comments that

control of soil-borne diseases is of great economical importance but no attempts at biological control have yet been successful. Katznelson, Lockhead and Timonin (19) cite numerous instances of excretions, such as that produced by the young legume root which attracts the nodule bacteria to the root. Lockhead (20) was prompted to write that "Resistance to a certain disease may be linked up with a selective action of root excretions upon the saprophytic soil microflora, thus favoring types which may be more and in other cases, less antagonistic (directly or indirectly) towards pathogenic organisms". Timonin (40) demonstrated that a variety of flax resistant to root rot, excreted hydrocyanic acid into the surrounding medium, thus exercising a selective power upon the fungus flora of the rhizosphere. The cyanamide depressed certain pathogenic fungi such as *Fusarium* and *Helminthosporium* but appeared to favor the growth of *Trichoderma viride*, an organism frequently mentioned for its ability to retard other fungi. Susceptible varieties of flax on the other hand produced by-products that stimulated the growth of the pathogens mentioned. According to Katznelson et al. (19), Thom has pointed out that it is difficult to distinguish between substances actually excreted from the roots and the products of decomposition of root and plant fragments.

Cochrane (4) has proposed that some root rots are



initiated by the direct toxic action of plant residues, and he implies that the activities of the soil organisms are secondary and incident to an initial injury that is chemical in origin. Using a seedling injury technique, he demonstrated that a water extract of plant tissue of ladino clover and perennial rye grass produced seedling injury typical of root rot, while plant tissue of soybean and corn produced no injury.

Study of more than 300 references to the peppermint plant and its oil yielded no direct information on the effect of this plant on cabbage or the clubroot disease. A few references to the effect of the peppermint oil and to menthol, one of its constituents, on bacteria and fungi are enlightening and are included as related material.

Stewart and Howe (38) have shown that oil-secreting glands are found on stolons of the peppermint plant as well as on the parts of the vertical shoot. In the growing points of both stolon and vegetative shoot, little anatomical difference could be observed in relation to the oil glands. These oil-secreting glands appear to be epidermal in origin. They develop from a conspicuously large cell which divides to form a stalk cell and an upper cell. The latter gives rise to the capitulum which consists of eight secretory cells. The secretory cells of the mature gland are contained within a cup-shaped and presumably cellulose membrane outside of which the oil seems to form.

The oil droplet also seems to be surrounded by a distended membrane. Oil glands are not found on the roots of the plant.

Meyers and Thienes (26) working with certain volatile oils, including peppermint, found that an aqueous solution of peppermint oil killed yeasts in approximately 100 hours. Meyers (25) later showed that certain volatile oils had fungicidal value. A saturated aqueous solution of peppermint or menthol killed yeast in about 120 hours, but bacteria, according to Meyers, are not seriously inhibited.

According to Pacheco and Costa (27), menthol effects the lysis (mentholysis) of many species of bacteria. There are genera of bacteria which are sensitive and others that are not. Within some species, there are individual specimens that are sensitive or not to mentholysis. Other terpenes, such as camphor and eucalyptol, do not effect the lysis of bacteria.

Webb (51) reviewed the use of essential oils as anti-septics and noted that Koch, 1890, was of the opinion that the germicidal action of peppermint oil was comparable to that of a 1:2500 dilution of copper sulfate. Von Behring rejected the use of essential oils as disinfectants. Other workers suggested the use of peppermint oil in genito-urinary infections, sterilization of catgut and as a prophylactic against cholera. Oil of peppermint is used in root-canal dressings in dentistry, advantage being

taken of its analgesic effect as well as any antiseptic properties that it may possess. Webb and Tanner had previously reported that a 0.1% concentration of peppermint, wintergreen, bay, clove, mustard, or cinnamon oil completely inhibited growth of yeasts. Oils of almond, ginger, nutmeg, peppers, lemon, onion and vanilla did not inhibit yeasts at the 0.1% concentration, but all oils were effective at 10% concentration.

## EXPERIMENTAL METHODS AND RESULTS

This study has been carried out at the Northern Indiana Muck Crops Experimental Farm, Walkerton, Indiana, in the greenhouses of Purdue University, and in the greenhouses of Michigan State College. The soil used in the greenhouse studies was taken from the same area that was used in the field trials. The muck soil is a Carlisle type with a reaction of pH 5.2. It is a well-decomposed muck that is well drained during the growing season, but that was at one time subject to overflow during winter and spring. Dikes were constructed in 1947 and 1948 that prevented the area from being flooded in the growing seasons of 1947, 1948 and 1949.

The clubroot organism, P. brassicae was unknowingly introduced into the soil on the Northern Indiana Muck Crops Experimental Farm in 1938. Spores of the organism were apparently carried in soil brought to the farm with a load of one-year-old peppermint stolons that were used for establishing a new peppermint planting on the farm. The planting stock and soil were unloaded in a roadway adjacent to the field. Clubroot infected cabbage was observed the first summer in a planting 30 feet away from this initial point of introduction. Isolation of the area was attempted, but each season more infected plants were found at various places on the 60 acre tract of land. After a spring flood in 1943, which covered the entire

area, the soil was so uniformly contaminated that cabbage production was abandoned.

#### EXPLORATORY EXPERIMENTS ON CONTROL OF CLUBROOT

##### Soil fumigants

In the spring of 1945, pot trials were set up in the greenhouse to test the fungicidal and phytotoxic effects of two soil fumigants on the clubroot organism and the cabbage and mint plants. The soils were treated with the chemicals and compared to steam sterilized muck and to soil that was not treated. The treatments were in duplicate for each crop. The muck soil was thoroughly mixed before it was weighed and placed in two-gallon pots. The pots to be steam sterilized were then treated in a retort at 250° F for 1 hour. The fumigants, methyl bromide and dichloropropene-dichloropropane were applied by dropping 2 ml. of the liquid in a hole made in the center of the soil in each treated pot. This treatment approximated 35 gallons of fumigant per acre. Cabbage was planted in two pots of each of the treatments and peppermint in each of the other two. The mint grew well in all pots and no differences were observed. The cabbage, on the other hand, died in the steam sterilized pots, perhaps due to a form of toxicity commonly observed following sterilization in which nitrates are commonly converted to nitrites. In the pots that received no treatment or had been treated with either of the fumigants, the cabbage started well

but later all became infected with clubroot. The infected plants were then pulled and destroyed. The pots remained in the greenhouse during the summer, the mint matured and was left in the pots to dry. In the spring of 1946, the soil of the pots was prepared for planting again. The mint was worked into the soil, and all pots were planted to cabbage. Table 1 gives the results of this trial in percentage of roots infected with clubroot in each treatment.

Cabbage free of clubroot was produced on all pots in which mint had been grown in 1945. In the pots in which cabbage had been grown, however, the steam sterilized soil was the only one on which club-free plants were produced. From these meager data, it appears that neither of the soil fumigants effected control of the organism, but there was a good indication that the peppermint had produced some residual effect in the soil that reduced the amount of clubbing on the cabbage that was grown subsequently on the soil.

#### Calcium Cyanamid

Calcium Cyanamid has been used by several workers (18) for the control of clubroot, with variable results. In 1945, duplicate plots of the following quantities of Calcium Cyanamid were set up on contaminated soil of the Purdue Muck Crops Farm:

Table 1.

Effect of two soil fumigants, steam sterilization  
and growth of peppermint on persistence of the  
clubroot organism in muck soil

Greenhouse, Lafayette, Indiana

| Pot<br>No. | 1945<br>treatment | 1945<br>crop | Percent cabbage (1946)<br>roots infected with clubroot |
|------------|-------------------|--------------|--------------------------------------------------------|
| 1          | Control           | Mint         | 0                                                      |
| 2          | Steam             | Mint         | 0                                                      |
| 3          | Methyl bromide    | Mint         | 0                                                      |
| 4          | D.D.*             | Mint         | 0                                                      |
| 5          | Control           | Mint         | 0                                                      |
| 6          | Steam             | Mint         | 0                                                      |
| 7          | Methyl bromide    | Mint         | 0                                                      |
| 8          | D.D.              | Mint         | 0                                                      |
| 9          | Control           | Cabbage      | 33                                                     |
| 10         | Steam             | Cabbage      | 0                                                      |
| 11         | D.D.              | Cabbage      | 31                                                     |
| 12         | Methyl bromide    | Cabbage      | 60                                                     |
| 13         | D.D.              | Cabbage      | 71                                                     |
| 14         | Methyl bromide    | Cabbage      | 33                                                     |
| 15         | Steam             | Cabbage      | 0                                                      |
| 16         | Control           | Cabbage      | 57                                                     |

\* dichloropropene-dichloropropane

1. No Cyanamid applied
2. 500 pounds Cyanamid per acre
3. 1000 pounds Cyanamid per acre
4. 2000 pounds Cyanamid per acre

The treatments were applied by opening a furrow about 6 inches deep for each row. The specified amount of Calcium Cyanamid was then applied in several applications and the soil was gradually drawn in to fill the furrow as each application was made. An excessive application was made in order to test the effectiveness of the treatment, regardless of the cost. The furrow was packed down and allowed to stand for two weeks before planting. Cabbage was direct seeded in the rows with a hand seeder on June 6th. Poor emergence of the seedlings was observed on the two highest rates of application and gradually all plants died on the 2000 pound treatment as well as many on the other treated plots. On July 9, these plots were reseeded and many plants of the second crop also died. Those that remained were pulled and examined in October and all roots were found to be infected with clubroot. Since this soil was acid in reaction, these results may be explained by the fact that urea was probably released rather than dicyanodiamide (5) which is reported to be the effective agent, released under alkaline soil conditions, which may kill the clubroot organism.



### Varietal resistance

Walker (48) reported that cabbage-kale rogue crosses, found in a Wisconsin field in 1941, were resistant to clubroot. To test the possibility of some resistance being present in one of the commercial varieties adapted to Northern Indiana, a variety trial was planted on land suspected of being contaminated with the clubroot organism. Neither cabbage nor peppermint had been grown on this soil previously but the soil had been flooded. The list of varieties and degree of infection in each plot is presented in Table 2. The complete data are shown to emphasize the variability of the results. No differences in resistance were found between these varieties. It is apparent that low incidence of infection on any one plot can only be attributed to light contamination in the area or to escape from infection, and not to differences in resistance between varieties.

### Potassium

The effect of potassium in increasing the incidence of infection of cabbage roots by the clubroot organism was studied by Pryor (30), Gries (13) and Walker (46). In controlled experiments, these workers have demonstrated that increased amounts of potassium will also increase the amount of clubbing. If, in field practice, this relationship held true, it would be impractical to recommend high potash fertilizers for cabbage on soils known

Table 2.

Relative reaction of certain common cabbage varieties  
to infection by P. brassicae Wor.

Walkerton, Indiana 1947

| Variety<br>Number | Variety               | Percent infected plants |           |           |      |
|-------------------|-----------------------|-------------------------|-----------|-----------|------|
|                   |                       | Rep.<br>1               | Rep.<br>2 | Rep.<br>3 | Ave. |
| 1                 | Cornell Early Savoy   | 70.5                    | 0         | 18        | 29.5 |
| 2                 | Ferry's Hollander     | 58                      | 5.5       | 33.3      | 32.3 |
| 3                 | Marion Market         | 53                      | 50        | 77        | 60   |
| 4                 | Early Seneca          | 41.7                    | 63.7      | 32.8      | 46.1 |
| 5                 | Penn State Ballhead   | 20                      | 4.7       | 37.5      | 20.7 |
| 6                 | Wisconsin All Seasons | 77.6                    | 17.6      | 9.7       | 34.9 |
| 7                 | Enkhuizen             | 46.2                    | 12.5      | 52.9      | 37.2 |
| 8                 | Mid Season Market     | 45                      | 46.8      | 33.3      | 41.7 |
| 9                 | Globe                 | 24.6                    | 11.5      | 83.5      | 39.8 |
| 10                | Copenhagen Market     | 38                      | 78.3      | 60.4      | 59.2 |
| 11                | Allhead Select        | 25.6                    | 75.3      | 97.5      | 66.1 |
| 12                | Allhead Early         | 26.9                    | 64.8      | 88.8      | 60.2 |
| 13                | Bugner                | 22.8                    | 19.7      | 59.4      | 33.9 |
| 14                | Wisconsin Hollander   | 15.3                    | 69        | 31        | 38.8 |

No significance

Plot size -- 12' x 50' -- 2 center rows harvested for count

Fertilizer 0-9-27 -- 1000# per acre

to be contaminated with P. brassicae. To test the effect of potassium on the clubroot organism in muck soil, plots were established at the Walkerton Farm. These plots were to

determine if potash in large quantities, under field conditions, would actually increase the amount of clubroot. The plot area was prepared by plowing and discing. One hundred pounds of  $P_2O_5$  was applied uniformly over the area. Each plot in the series was 40 x 70 feet and four replications of the following treatments were set up:

1. No additional  $K_2O$
2. 100 pounds  $K_2O$  per acre
3. 500 pounds  $K_2O$  per acre
4. 1000 pounds  $K_2O$  per acre

Cabbage of the All seasons variety was seeded in the plots on June 1. The cabbage grew well and when harvested in October none of the roots on any of the plots were infected with the clubroot organism. This was completely contrary to any expected results and consequently the cropping history of this area was studied. It was found that the land had been in peppermint for the three years preceding the crop of cabbage. Further studies on the effect of potassium on the incidence of clubroot were abandoned in favor of studying the effect of mint crops, in rotation with cabbage, on the subsequent development of clubroot on the cabbage.

#### EFFECT OF PEPPERMINT ON THE PERSISTENCE OF THE CLUBROOT ORGANISM

Greenhouse trials with soils taken from field plots established in 1946

Field plots were laid out in the spring of 1946 on

soil on which all cabbage died of clubroot in 1945. Three replications of split plots were used so that cabbage could be grown each year from 1946 to 1949 with the arrangement such that cabbage would be grown on soil that previously had been cropped to cabbage, and to mint for one, for two and for three years in succession. The plot size was 30 x 100 feet. The English or Black peppermint, Mentha piperita L., was used to plant the mint plots. Stolons for planting were taken from a one-year-old stand of mint on the farm. Growth was good, and when the mint reached the proper size for harvesting half of each plot was cut and distilled. The yield was at the rate of 45 pounds of oil to the acre. The mint on the other half of each plot was left to stand until it was killed back by frost. All of the cabbage in the plots died before harvest as a result of infection by the clubroot organism.

In November of 1946, soil was taken from the field representing the treatments shown in Table 3. The soil was carried in individual sacks to the greenhouse, where it was put in 2 $\frac{1}{2}$ -inch deep flats and planted to cabbage. Menthol normally makes up about 50% of the chemical constituents of peppermint oil and considering that some oil is lost on the soil from a crop, additional tests (treatments 6 and 7) were included in the trial. The menthol was added in quantities that would be equivalent to several years accumulation from continuous crops of

Table 3.

Effect of (1) one year of certain cultural practices with peppermint in the field; and  
 (2) the addition of menthol to contaminated soil in the greenhouse,  
 on the infection of cabbage plants with P. brassicae

Lafayette, Indiana

| Treatment                                                  | Clubroot infected cabbage plants |                    |
|------------------------------------------------------------|----------------------------------|--------------------|
|                                                            | Percent<br>each flat             | Average<br>percent |
| 1 Control-contaminated soil                                | 67, 15, 20, 47                   | 37                 |
| 2 Soil with mint tops and roots removed                    | 80, 20, 93, 0                    | 48                 |
| 3 Mint harvested at regular harvest period                 | 33, 92, 33, 53                   | 52                 |
| 4 Mint cut and removed at time of sampling                 | 66, 76, 0, 0                     | 35                 |
| 5 Mint not cut, tops turned in                             | 40, 46, 0, 81                    | 42                 |
| 6 Menthol added, 1 gm. per flat<br>(approx. 50# per acre)  | 9, 20, 33, 13                    | 17                 |
| 7 Menthol added, 2 gm. per flat<br>(approx. 100# per acre) | 33, 7, 15, 7                     | 15                 |
| 8* 2 years of <u>Mentha arvensis</u><br>in adjacent field  | 9, 0, 0, 0                       | 0                  |

No significance

\*Treatment 8 was not included in the statistical calculations because the treatment was on an adjacent field rather than on the plot area.

peppermint. It was dissolved in 95% ethyl alcohol and diluted further with water. The liquid was mixed thoroughly with the soil in the flat. Even though the trend seems to

indicate that the menthol decreased the number of infected plants, statistical analysis of the data shows that the differences were not significant.

A possible source of error, and one corrected in the next field experiment, was introduced by the wide row spacing. The planting had been made in rows 36 inches apart, which is the standard spacing in commercial production. This left much bare soil between rows of mint and even some soil that was covered in the fall by stolons had not been in contact with the mint plant during the major part of the growing season. It was impossible in sampling to know for sure that the soil that was sampled had actually received the treatment ascribed to it.

Variation such as that exhibited by treatment 4 in Table 3, no doubt, can be attributed to sampling procedures on the wide spacing. Treatment 8 was not considered in the statistical analysis because the soil had not been definitely tested for the presence of the clubroot organism although it had been flooded in previous years and was adjacent to land known to be contaminated. This soil was taken from an area on which Mentha arvensis var. piperescens Malv., often called Japanese mint, had been grown for two seasons. Mentha arvensis is not as hardy as the peppermint of commerce and the results are not presented here because of any commercial possibilities for the crop in the Midwest. The field plots from which all soil

samples were taken in 1946, were destroyed in May of 1947 by flooding and erosion across the west half of the area.

Field and greenhouse experiment 1947 - 1950

Following the loss of the first series of plots which had been designed to test the mint-cabbage clubroot relationship, a second series was established to the east and adjoining the first. Four replications of each treatment were so arranged that the data in the last year could be analyzed as a latin square. In other years, when only two and three plots had cabbage growing on them, the data were treated as replicated randomized blocks.

The first plots were 30 x 100 feet in size, but in order to get more uniform soil and to add one more replication, the plot size was reduced in 1947, and subsequent years, to 12 x 20 feet. More careful operations were required on the smaller plots and a greater opportunity for recontaminating a plot was presented by the use of tools such as the rotary tiller and seeder. It was also difficult to keep the mint plants from spreading into the adjoining cabbage plots.

Peppermint plants were used to set these plots instead of stolons. This was necessary because these plots were established in June, after a flood in late May, and no stolons were available at that time. The plants were set on 12 inch centers and, in most plots, a fair growth was made which covered the soil. The mint was not cut and by

fall it had formed a mat on the surface of the plot. The plots that were not in mint in 1947, were fallowed. The field plan, Figure 1, of these plots is outlined for reference in discussing Tables 4 to 10.

#### Effect of one year of mint - 1948

Greenhouse pot tests were used to supplement the field trials because the cabbage could be grown under conditions in which the moisture could be controlled, thereby providing optimum conditions for infection by the organism. In March, 1948, 4 individual samples of soil were taken from each of two plots in each of the four replications of the field plots for greenhouse studies. One plot that had not grown any crop in 1947 and one on which peppermint had been grown, constituted each pair. The numbers of the plots given in the tables are for reference to the plot design (Fig. 1). The results of the trial in the spring of 1948 are given in Table 4. Infestation is based on 7 to 13 plants per pot, representing 34 to 44 plants per treatment. It will be observed from these data that all plants grown on soil from the plots that were fallowed in 1947, were infected. These data indicate that the growing of mint for only one year resulted in less club-root on the cabbage plants grown on this soil the following year.

Cabbage was seeded in the designated field plots in the spring of 1948, after the soil had been fitted by





|         |      |         |    |         |    |         |    |
|---------|------|---------|----|---------|----|---------|----|
| 1       |      | 5       |    | 9       |    | 13      |    |
| Mint    | 1947 | Fallow  | 47 | Mint    | 47 | Mint    | 47 |
| Mint    | 48   | Cabbage | 48 | Mint    | 48 | Cabbage | 48 |
| Cabbage | 49   | Cabbage | 49 | Mint    | 49 | Cabbage | 49 |
| Cabbage | 50   | Cabbage | 50 | Cabbage | 50 | Cabbage | 50 |
| 2       |      | 6       |    | 10      |    | 14      |    |
| Fallow  | 47   | Mint    | 47 | Mint    | 47 | Mint    | 47 |
| Cabbage | 48   | Mint    | 48 | Cabbage | 48 | Mint    | 48 |
| Cabbage | 49   | Cabbage | 49 | Cabbage | 49 | Mint    | 49 |
| Cabbage | 50   | Cabbage | 50 | Cabbage | 50 | Cabbage | 50 |
| 3       |      | 7       |    | 11      |    | 15      |    |
| Mint    | 47   | Mint    | 47 | Mint    | 47 | Fallow  | 47 |
| Cabbage | 48   | Mint    | 48 | Mint    | 48 | Cabbage | 48 |
| Cabbage | 49   | Mint    | 49 | Cabbage | 49 | Cabbage | 49 |
| Cabbage | 50   | Cabbage | 50 | Cabbage | 50 | Cabbage | 50 |
| 4       |      | 8       |    | 12      |    | 16      |    |
| Mint    | 47   | Mint    | 47 | Fallow  | 47 | Mint    | 47 |
| Mint    | 48   | Cabbage | 48 | Cabbage | 48 | Mint    | 48 |
| Mint    | 49   | Cabbage | 49 | Cabbage | 49 | Cabbage | 49 |
| Cabbage | 50   | Cabbage | 50 | Cabbage | 50 | Cabbage | 50 |

Fertilizer 0-10-20 -- 1000# per acre

Plot size -- 12' x 20'

Figure 1. Design of field plots laid out in 1947.

The crop and year it was grown are  
shown for each plot.

Walkerton, Indiana

Table 4.

Effect of growing peppermint for one year  
compared with one year of fallowing,  
on the infection of cabbage plants with clubroot

Greenhouse Experiment -- Spring 1948

| Rep. | Plot<br>Number | Percent<br>Clubbed<br>Plants | Plot<br>Number | Percent<br>Clubbed<br>Plants |
|------|----------------|------------------------------|----------------|------------------------------|
| 1    | 2              | 100<br>100<br>100<br>100     | 3              | 0<br>0<br>0<br>0             |
| 2    | 5              | 100<br>100<br>100<br>100     | 7              | 10<br>0<br>0<br>0            |
| 3    | 12             | 100<br>100<br>100<br>100     | 9              | 85<br>81<br>87<br>77         |
| 4    | 15             | 100<br>100<br>100<br>100     | 16             | 14<br>25<br>72<br>0          |

running through it with a rototiller. This operation was necessary in order to work the soil in preparation for planting, but it also may have resulted in the recontamination of the soil. All barren areas in the plots that were scheduled to remain in mint were filled in by hand planting.

Table 5 gives the percentage of infected cabbage plants on the field plots, based on a population of 53 to 64 plants. These data are significant at the 5% level

Table 5.

Effect of growing peppermint for one year  
preceding the cabbage crop,  
on the incidence of clubroot

Walkerton, Indiana 1948

| Rep. | Fallow 1947 |                   | Peppermint 1947 |                   |
|------|-------------|-------------------|-----------------|-------------------|
|      | Plot No.    | Percent infection | Plot No.        | Percent infection |
| 1    | 2           | 72                | 3               | 22                |
| 2    | 5           | 100               | 8               | 13                |
| 3    | 12          | 98                | 10              | 76                |
| 4    | 15          | 36                | 13              | 19                |
| Ave. |             | 76.5              |                 | 32.5              |

L.S.D. 38.5

Difference is significant at 5% level

Plots harvested August 5, 1948

and agree with the data in Table 4 obtained from the preceding greenhouse study. Original data are again shown here, along with plot numbers, for ready reference to the plot plan. These data show a high percentage of infected plants in plot 10 but the trend is for less contamination from P. brassicae in all plots after only one crop of peppermint. Variability in stand of mint the preceding year may account for some of the erratic results, but the high percentage of infection on plots 9 and 10 may be accounted for more readily by looking at Figures 2 and 3. These photos show plots 9 and 10 to be in a depression adjoining plot 5 on which only cabbage has been grown,

and which is severely contaminated with clubroot. Water standing in this depression would spread spores of P. brassicae and would reinoculate plots 9 and 10 to some extent each time it rained with sufficient intensity for water to stand on the surface. Soil barriers were laid up between plots in the fall of 1948 to partially prevent the spread of the clubroot organism by water run-off or by standing water.

#### Effect of two years of mint - 1949

On November 4, 1948, three plots from each replication were sampled to determine the effect of one and two years of mint on the persistence of the clubroot organism in the soil. Eight individual samples were taken from each field plot. The soil was taken to the greenhouse and was put in six-inch clay pots and seeded with 10 cabbage seeds. Generally, about 7 seeds germinated and grew making a total of 46 to 62 plants per treatment of eight pots. After harvest, the percentage of the infected plants was calculated and this figure was used in the analysis of the data presented in Table 6. These data show that there is a decrease in the number of plants attacked by clubroot after one year of peppermint, and that the beneficial effect has not been lost even after a crop of cabbage is grown on the same plots following the mint. The averages indicate a trend toward still further reduction of contamination after two years of peppermint but this reduction



Figure 2. View of field plots. Men are in plot 5.  
Plot 11 is in immediate foreground. Plot 10  
to the right shows slope of land toward  
plot 5.

Walkerton, Indiana



Figure 3. View of plot 10 showing that the land in the foreground of the photo is lower than that in the rear and that the cabbage plants in the foreground are wilted, indicating infection of the plant while those above the level of standing water are not infected.

Table 6.

Comparison of the effect of one and two years of  
peppermint on the persistence of the  
clubroot organism in a muck soil

Greenhouse trial 1949

| Rep. | <u>One year fallow</u><br><u>one year cabbage</u> |                      | <u>One year mint</u><br><u>one year cabbage</u> |                      | <u>Two years mint</u> |                      |
|------|---------------------------------------------------|----------------------|-------------------------------------------------|----------------------|-----------------------|----------------------|
|      | Plot<br>No.                                       | Percent<br>infection | Plot<br>No.                                     | Percent<br>infection | Plot<br>No.           | Percent<br>infection |
| 1    | 2                                                 | 69                   | 3                                               | 13                   | 1                     | 6                    |
| 2    | 5                                                 | 86                   | 8                                               | 0                    | 7                     | 7                    |
| 3    | 12                                                | 98                   | 10                                              | 63                   | 11                    | 26                   |
| 4    | 15                                                | 60                   | 13                                              | 2                    | 16                    | 0                    |
| Ave. |                                                   | 72.2                 |                                                 | 19.5                 |                       | 9.7                  |

L.S.D. 20.8

Difference is significant at 1% level

was not within the limits of the L.S.D. of the experiment. It should be pointed out, however, that on two plots the contamination approached zero after only one year of peppermint. It would, therefore, be impossible to show statistically a difference between one and two years of mint after such a low point of infection was reached.

When the cabbage plants had been removed on August 5, 1948, a further treatment was applied. This treatment would test the effect of the addition of green mint plant to a soil, which had not previously had mint on it, in order to determine if control of P. brassicae by peppermint was affected by the growing of the plant on the soil or

by the addition of the green plant material to the soil.

The 8 plots that had produced cabbage plants in 1948 were split, and to the half of the plot to the north, chopped mint hay, stolons, and roots were applied in an amount equivalent to that grown on an area of the same size. In fact, an area of equal size was measured in an adjacent field of mint and the roots, stolons, and tops, removed and chopped in an ensilage cutter. The shredded plant material was then spread on the soil and worked into the surface with rakes. Cabbage seed was sown in the plots thereafter. This seeding was done late in the season of 1948 and the plants failed to grow. The plots, consequently, overwintered without further treatment.

In the spring of 1949, the plots that were to be planted to cabbage were prepared by running through them several times with a Tillivator to tear up the mint refuse and roots. On May 5th, cabbage seed of the Marion Market variety was drilled in the plots with a hand seeder. Two rows were seeded 36 inches apart and about 18 inches on either side of the center of the plot. When the cabbage plants were about 12 inches tall, (July 20) they were thinned by hand pulling to allow one plant to remain for each 12 to 15 inches of row. The spacing could not be exact as the plants had to be left in place because a clubbed plant cannot be transplanted successfully.



The data from each row of every plot were kept separate because one row of each of the plots cropped to cabbage in 1948 had had the green peppermint plant material added in the fall of 1948. Tables 7, 8\* and 9 were prepared from data obtained when thinning the plots on July 20 and from harvesting the plots on October 5. The percentages of infected plants on July 20 from the whole plots (both rows) are shown in Table 7. The data from each of the plots are given as well as the average of each treatment for all replications. The difference between treatment 1 and treatments 2 and 3 is significant at the 1% level. Treatment 1 represents the plots fallowed in 1947 on which cabbage was grown in 1948; these plots averaged 69.4 percent infected plants. Treatment 2 had mint grown on it in 1947 followed by cabbage in 1948 while treatment 3 had been in mint for two years. Treatments 2 and 3 averaged only 15.2 percent of infected plants. No difference was found between one and two years of mint in this experiment and it is obvious again that differences would be difficult to obtain when, after one year of mint, the contamination from P. brassicae approached zero. It will be noted by reference to the data for treatment 3 (two years of peppermint) in Table 7 that there was a tendency on some plots for more infection

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\*The details of the analysis of variance for the data in Tables 7 and 8 are found in Appendix I.



Figure 6. Plants pulled from the field July 20, 1949.

The three on the left are normal plants from plot 1 which had been in peppermint for two years preceding the crop of cabbage. These plants are normal, and free of clubroot. The plants on the right are typical of those on plot 2. These plants are stunted, and each root system is a clubbed mass of roots. Each day a plant so infected will wilt in the field.

Table 7.

Clubroot infection of cabbage in plots on  
which mint had been grown 0, 1 and 2 years

Walkerton, Indiana 1949

| Rep.              | Treatment |    |         |    |         |    |
|-------------------|-----------|----|---------|----|---------|----|
|                   | Fallow    | 47 | Mint    | 47 | Mint    | 47 |
|                   | Cabbage   | 48 | Cabbage | 48 | Mint    | 48 |
|                   | Cabbage   | 49 | Cabbage | 49 | Cabbage | 49 |
| Percent infection |           |    |         |    |         |    |
| 1                 | 76.1      |    | 3.6     |    | 8.1     |    |
| 2                 | 92        |    | 1.2     |    | 12.5    |    |
| 3                 | 86.5      |    | 56.2    |    | 30.7    |    |
| 4                 | 46.1      |    | 0       |    | 9.4     |    |
| Ave.              | 69.4      |    | 15.2    |    | 15.2    |    |

Difference is significant at 1% level

from clubroot on soil that had been cropped to mint for two years than when only one year of mint had been grown but it has already been pointed out that this difference is not significant. Mention should be made, however, that the plots that had been in mint for two years required additional mechanical manipulation and traffic in their preparation, and it is supposed that some recontamination resulted. Such an occurrence could account for the slight increases in infection which appear in replications one, two and four.

### Effect of applying chopped green mint plants

Chopped plant material previously mentioned, was applied to plots on which cabbage was grown in 1948. The results of the effect of this material on reducing the contamination are presented in Table 8. Paired plots in the replications are shown with the original data reduced to percentage of cabbage plants infected. From these data, based on the plants harvested July 20, it appears that the application of the chopped plant material had no effect on reducing contamination by P. brassicae either on soil that had only had cabbage growing on it or on soil that had

Table 8.

Application of chopped green peppermint plants to a clubroot-contaminated soil, and its effect on the persistence of *P. brassicae*

Walkerton, Indiana 1949

|                 |                      | Treatment                  |                      |                            |                      |                               |                            |
|-----------------|----------------------|----------------------------|----------------------|----------------------------|----------------------|-------------------------------|----------------------------|
|                 |                      | Fallow 47<br>Cabbage 48    |                      | Mint 47<br>Cabbage 48      |                      | All plots                     |                            |
|                 |                      | Plant<br>material<br>added |                      | Plant<br>material<br>added |                      | No plant<br>material<br>added | Plant<br>material<br>added |
| Rep.            | Percent<br>infection | Percent<br>infection       | Percent<br>infection | Percent<br>infection       | Percent<br>infection | Percent<br>infection          | Percent<br>infection       |
| 1               | 62.5                 | 89.7                       | 0.0                  | 7.1                        |                      |                               |                            |
| 2               | 87.9                 | 96.1                       | 0.0                  | 2.3                        |                      |                               |                            |
| 3               | 86.7                 | 86.3                       | 54.5                 | 57.9                       |                      |                               |                            |
| 4               | 26.8                 | 19.3                       | 0.0                  | 0.0                        |                      |                               |                            |
| Ave.            | 65.9                 | 72.8                       | 13.6                 | 16.8                       |                      | 39.8                          | 44.8                       |
| No significance |                      |                            |                      |                            |                      |                               |                            |

grown one crop of mint and one crop of cabbage. The average of all plots which had only cabbage on them, shows that the half-plots that received the additional plant material tended to produce cabbage with more clubroot infection than the half-plot without. The tendency was the same for all plots whether those plots had been cropped to mint or cabbage for one year each. When all plots, regardless of previous treatment, but receiving the applications of chopped green mint plant material are compared with all plots not receiving this application of chopped mint plant the averages showed that 39.8 percent of the plants were infected by P. brassicae where no chopped green mint was added, although 44.8 percent of the plants were infected when chopped green plant material was added. This difference may indicate that the reduction in contamination is associated directly with the growth of the crop on the soil rather than with the presence of green plant material.

Table 9 summarizes the results of the 1949 field trial. The data includes the yield of mature cabbage, number of marketable heads, and percentage of plants infected by the clubroot organism. Each row of cabbage was harvested separately in order to get data on the effect of adding the green plant material to the soil, but inasmuch as there was no statistically demonstrable effect produced by adding the green chopped hay to the plots as shown in

Table 8, the data from both halves of each plot were totaled and analyzed as a single plot instead of a split plot. In treatment 1, which was fallowed in 1947 but cropped to cabbage in 1948, 86 percent of the plants were attacked by the clubroot organism. When, however, peppermint was grown in 1947 and cabbage in 1948, the infection was reduced to 30 percent. This difference is significant at the 1% level and agrees with the data in Table 7, which indicated a significant drop in number of plants attacked by the organism after one year of peppermint. Here again, however, no greater average drop in number of infected plants resulted from growing peppermint for two years compared with growing it for one year. From a statistical point of view this is in agreement with the findings in Tables 6 and 7, although the trend in Table 6 was for further decrease in the number of infected plants after two years of mint.

The number of marketable heads of cabbage on each plot averaged from 10.2 for the plots which had never had mint on them to 22 and 19.5 heads per plot for those having one and two years of peppermint preceding the cabbage. This difference in number of heads is significant at the 5% level but no difference was found between plots of one and two years of peppermint.

The two columns on the right side of Table 9 show the yield in pounds of cabbage per plot and the calculated

Table 9.

The effect of clubroot on the average yield\* of cabbage grown on soils that had previously been cropped to 0, 1, or 2 years of peppermint

Walkerton, Indiana 1949

| Treatment |                                | Cabbage plants attacked by <i>P. brassicae</i> | Marketable heads per plot | Yield per plot | Yield per acre |
|-----------|--------------------------------|------------------------------------------------|---------------------------|----------------|----------------|
|           |                                | Percent                                        | Number                    | Pounds         | Tons           |
| 1         | Fallow 47<br>Cabbage 48        | 85.5                                           | 10.2                      | 37             | 6.66           |
| 2         | Peppermint 47<br>Cabbage 48    | 30.0                                           | 22.0                      | 131            | 23.58          |
| 3         | Peppermint 47<br>Peppermint 48 | 30.7                                           | 19.5                      | 121            | 21.78          |
|           |                                | L.S.D. 1%                                      | L.S.D. 5%                 | L.S.D. 1%      | L.S.D. 1%      |
|           |                                | 10.6                                           | 9.2                       | 40.3           | 7.2            |

\*Each figure is the average of 4 replications

yield in tons per acre. The difference in yield is highly significant between the treatment which had no mint in the rotation and the treatments with one or two years of peppermint grown on the plot. The yield from the plots on which no mint had been grown was 6.6 tons per acre whereas the yields on the plots where one and two years of peppermint preceded the cabbage crop were 23.5 and 21.7 tons per acre respectively of marketable cabbage.

Effect of three years of mint - Greenhouse 1950

In the fall of 1949, the entire field plot area was

plowed to a depth of 8 inches with a regular moldboard plow. These plots will all be planted to cabbage in 1950 to complete the cycle of cropping as planned and outlined in Figure 1. Greenhouse trials have supplemented the field data in all cases in previous years, and in order to obtain data on the effect of 3 years of continuous mint on the persistence of the clubroot organism, samples of soil were taken from the plots to the greenhouse on February 9, 1950. Four pots representing each plot were planted to cabbage of the Golden Acre variety on February 11. The pots were watered frequently to make conditions favorable for infection, assuming that the organisms were present. The plants were removed from the pots and the infected plants counted on March 20. The data showing the amount of plant infection are presented in Table 10. Six plants were grown in each pot, making a total of 24 plants per treatment. The minimum infection was 70.8 percent from any block of plants representing continuous cabbage. In the soil from the mint plots, on the contrary, only 4 percent of the plants were found to be infected. Actually, 81 of 96 plants following cabbage were attacked whereas only 2 of 96 plants following 3 years of peppermint were attacked. The significance of the data is obvious without statistical calculation.



Table 10.

The effect of three years of continuous cropping to  
peppermint on the persistence of clubroot of  
cabbage in muck soil

Greenhouse 1950

| Rep. | Treatment                      |                               |                |                               |
|------|--------------------------------|-------------------------------|----------------|-------------------------------|
|      | Fallow 1 yr.<br>Cabbage 2 yrs. |                               | Mint 3 yrs.    |                               |
|      | Plot<br>number                 | Percent<br>infected<br>plants | Plot<br>number | Percent<br>infected<br>plants |
| 1    | 2                              | 75.0                          | 4              | 0.0                           |
| 2    | 5                              | 70.8                          | 7              | 4.0                           |
| 3    | 12                             | 91.6                          | 9              | 0.0                           |
| 4    | 15                             | 100.0                         | 14             | 4.0                           |
| Ave. |                                | 84.4                          |                | 2.0                           |

## DEPTH AT WHICH SPORES WERE FOUND IN MUCK SOIL

Spores of P. brassicae have been found at various depths in the soil. Wilson (55), in Ohio, reported finding them down to 12 inches in muck. Spore depth is important in view of the shallow nature of the root system of the mint plant and the fact that in normal cultural procedures, the peppermint is plowed under in the fall. The depth to which the mint material is turned under could be varied, if mint is to be grown in rotation with cabbage and the plant refuse or living plant is proven to be a factor in clubroot control.

In the fall of 1946, flats were prepared from soil that represented samples taken at two depths from the areas that had been in cabbage on the field plots that had been established the previous spring. All cabbage plants were attacked by clubroot when grown in soil taken at a depth of 4 to 8 inches. In soil taken at the 8 to 12 inch level 70 and 80 percent of the cabbage plants were attacked showing that many spores were present in the 8 to 12 inch layer.

In the spring of 1948, samples were taken from the alleyways around the new field plots and from the east end of the field that had been a part of the plots which had been in mint in the spring of 1946 and had remained in mint after the plots had been abandoned because of the flooding of the west portion of these plots in the spring

of 1947. Table 11 shows the results of growing cabbage in these pots of soil in the greenhouse. The soil samples had been taken in separate sacks to the greenhouse and potted carefully to avoid further contamination. These data show that in the soil adjacent to the field plots, and which could be considered representative of the general area, the spores were found only in the upper 8 inch level. In soil from the field from which samples had been taken two years previously and which had shown 100 percent contamination at that time, no clubs were formed on cabbage plants growing after 2 years of continuous cropping to peppermint. The latter soil had previously been contaminated to a depth of 12 inches. The mint on the field from which no infection took place had not been plowed down as in normal practice, and it was cut only in 1946. Soil samples were taken again from this same area in the spring of 1950 and as found previously, no clubs were produced on cabbage grown on them. Following the mint, this field had been in corn for the two years. From these data it would seem that the spores of the clubroot organism actually die out and are not merely inhibited in growth by the association with the mint crop. Further work should be done to determine if the depth of penetration of the spores will affect the time required to rid the soil of the clubroot organism.

Table 11.

Depth at which the clubroot organism was found  
in the muck soil of the farm

Walkerton, Indiana 1948

| Depth from<br>which soil<br>samples taken | Infection of plants<br>grown on soil taken<br>from alleys in field<br>plots | Infection of cabbage<br>plants grown on soil<br>taken from mint plots<br>planted in 1946<br>2 years of mint not<br>plowed |
|-------------------------------------------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|
| Inches                                    | Percent                                                                     | Percent                                                                                                                   |
| 0 - 8                                     | 50                                                                          | 0                                                                                                                         |
|                                           | 60                                                                          | 0                                                                                                                         |
|                                           | 80                                                                          | 0                                                                                                                         |
|                                           | 60                                                                          | 0                                                                                                                         |
| 8 - 16                                    | 0                                                                           | 0                                                                                                                         |
|                                           | 0                                                                           | 0                                                                                                                         |
|                                           | 0                                                                           | 0                                                                                                                         |
|                                           | 0                                                                           | 0                                                                                                                         |
| 16 - 24                                   | 0                                                                           | 0                                                                                                                         |
|                                           | 0                                                                           | 0                                                                                                                         |
|                                           | 0                                                                           | 0                                                                                                                         |
|                                           | 0                                                                           | 0                                                                                                                         |
| Planted May 15                            |                                                                             |                                                                                                                           |
| Harvested June 23                         |                                                                             |                                                                                                                           |

EFFECT OF CONTINUOUS CROPPING TO CABBAGE, MINT, OR CORN  
ON NITRATE AND AMMONIA PRODUCTION IN A MUCK SOIL

It has been shown that continuous cropping of a clubroot contaminated soil to peppermint will eliminate the clubroot organism. As an indication of selectivity of the effect of the mint plant on microorganisms, a determination of the nitrates and ammonia produced by incubation of soil samples in the laboratory, was made on soils which had been cropped to mint for three years, to corn for three years, and on a soil which had been fallowed for one year and on which cabbage was grown for two years.

Samples were taken from the field plots, already discussed, to represent the mint and cabbage rotations, and from the adjoining field of corn, which is shown to the left background in Figure 2. The samples, taken at four locations, represent four replications of each treatment. The soils were taken from the field on February 9, and put into six-inch pots. Moisture was held at the approximate field capacity before the determinations were made 16 and 51 days later. In the meantime the temperature was about 70° F or room temperature.

The methods used were those described by Peech and English (28) except that the samples were run on the moist soils. Moisture determinations were made at the time of analysis and the results were reported on the basis of oven dry soil. Samples were extracted with sodium acetate

solution buffered at pH 4.8. The brucine reagent was used for the colorimetric determination of nitrates and Nessler's reagent was used in the comparison of ammonia in the samples. Color comparisons were made by means of a photoelectric colorimeter and values were determined from a standard curve based on known quantities of ammonia and nitrate.

The amounts of ammonia and nitrates that developed under the different cropping systems, are presented in Table 12. Each figure in the table represents an average of four determinations on samples taken from four locations in the plot area. There is no significant difference between the treatments, and contrary to the belief held by growers that nitrification is inhibited by successive peppermint crops, there seems to be no trend of the plots cropped to mint to depress the ammonification or nitrification processes. It is suggested from these data that the effect of the mint on the soil is selective in that it does not inhibit the ammonifying and nitrifying bacteria, and yet eliminates P. brassicae from the soil microflora.

Table 12.

Effect of rotation on the production of  
nitrates and ammonia in a muck soil\*

| Treatment                        | <u>Nitrates in p.p.m.</u> |         | <u>Ammonia in p.p.m.</u> |         |
|----------------------------------|---------------------------|---------|--------------------------|---------|
|                                  | <u>Incubation period</u>  |         | <u>Incubation period</u> |         |
|                                  | 16 days                   | 51 days | 16 days                  | 51 days |
| 1 Fallow 1 yr.<br>Cabbage 2 yrs. | 87.5                      | 87      | 16                       | 21.7    |
| 2 Mint 3 yrs.                    | 88.7                      | 89.5    | 17.5                     | 23.7    |
| 3 Corn 3 yrs.                    | 80.5                      | 76.2    | 18.7                     | 19.5    |

No significance

\*Each figure represents the average of determinations  
from four locations

## DISCUSSION AND CONCLUSIONS

An observation made in 1945 indicated that clubroot of cabbage might be controlled by a crop rotation in which peppermint was grown for one or more years. Experimental data have been presented from tests that were set up to study this effect. These, in general, substantiate the observation.

Crop rotation has been stressed as an important measure in control of clubroot by many workers (18), but the time for suppression of the disease has varied from 3 to 8, or more, years. To be effective, any rotation must assure that no cruciferous plants, cultivated or wild, grow on the land. From this point of view alone, the peppermint crop would be excellent. Its growth habit tends to choke out many weeds. In addition, the quality of the commercial product depends on the fields being kept free of weeds so that no objectional odors or flavors are imparted by the weeds to the essential oil in distillation. The clubroot organism is an obligate parasite, and there is, therefore, little possibility that cruciferous weeds would be present in commercial mint fields to act as hosts for carrying over this organism.

The short time, in terms of crop successions, in which control of the organism was effected when the peppermint plant was grown on the muck soil used for the purposes of this study, indicates that the factors involved



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The short time, in terms of crop successions, in which control of the organism was effected when the peppermint plant was grown on the muck soil used for the purposes of this study, indicates that the factors involved

are more complex than simply the elimination of overwintering host plants.

It was shown in preliminary experiments that the common soil fumigants, methyl bromide and dichloropropene-dichloropropane had no effect on controlling the clubroot organism, and calcium cyanamide on this acid soil, although applied in quantities sufficient to kill the cabbage seedlings, did not kill the organism. None of the commonly grown cabbage varieties were found to be resistant to P. brassicae.

Throughout the investigation, numerous attempts to reproduce the field results were made in the greenhouse. Lucas (24), at the suggestion of the author, grew peppermint in  $2\frac{1}{2}$ -inch deep flats inoculated with the organism. After three months of favorable growth, the peppermint plants were cut in a Waring blender and mixed with the soil. Cabbage plants were planted later and none of them became infected with clubroot. Several trials in eight-inch and two-gallon pots were not successful when mint was grown for short periods of time (2 to 5 months) on soils naturally contaminated or inoculated with a suspension of clubroot spores. It is suggested that three factors may have been involved here: (1) the length of time given the mint plant to grow in these greenhouse trials, was much shorter than that taken in the field, (2) the relative depth of the soil in the pots in the greenhouse presents an abnormal situation in that the roots

of the mint plant did not penetrate to the bottom of the pots, yet the plant material was later mixed thoroughly with all of the soil, and (3) in the unsuccessful trials, cabbage plants were immediately transplanted into the treated soil without waiting for a period of time to elapse so that some activity could take place in the soil, which might result in the death of the clubroot organism. In greenhouse trials in which mint grew for a year, the organism, P. brassicae, was eliminated from the soil.

Data from the field plots show that when a crop of peppermint is grown on soil contaminated with clubroot, the organism is greatly reduced in population after only one year. It was not shown statistically that there was a further reduction of club-infected plants in the field after two years of mint. This was accounted for by the fact that some plots had zero infection after only one year of mint and, further, the possibility exists that recontamination took place when the rototiller used in the preparation of the plots traveled from the contaminated plots to the plots on which mint was grown.

Yield of marketable cabbage was increased from an average of 6.6 tons per acre when grown on contaminated soil, to 23.5 and 21.7 tons per acre when the cabbage was grown on soils cropped to mint for one and two years respectively. The percentage of infected plants was reduced from 85.5 percent on soil which had been cropped

only to cabbage, to 30 percent on the soil previously cropped to mint. It should also be pointed out that many of the plants in the latter group were only slightly infected and produced marketable heads although they were counted as infected plants. After three years of mint, the entire plot area was plowed in the fall with a mold-board plow and when cabbage was grown in samples of the soils taken from the plot areas the following spring, 100 percent of the cabbage plants were infected following cabbage, but no more than 4 percent were infected on any plot following three years of peppermint. No attempts were made to estimate the population of spores in the soil, but other workers (18) have claimed that the intensity of the infection depended on the concentration of spores. This factor coupled with the variability in stand of mint on the plots, would account for some of the variability in the recorded amount of reduction in infection -- higher populations of spores requiring a longer period of time to be eliminated. Along with the possible effect of concentration of spores in a soil on the amount of infection, is the consideration that the depth to which the spores penetrate would also be a factor. Trials on soil taken from the plots showed that P. brassicae spores were found at levels down to 8 and 12 inches in different areas of the plots but that the spores even at 12 inches were inactivated after two years of mint.

Six other species of mint, in addition to Mentha piperita, were grown in a short-time greenhouse experiment to determine if other species would be more effective than peppermint in the control of clubroot. In this experiment, the plant material was mixed into the soil and cabbage was planted immediately. Clubroot developed generally in all treatments, indicating that none of these species was much more effective than peppermint in effecting control of the disease.

Workers with essential oils and menthol (25,27,51) have shown that peppermint oil and menthol effect the lysis (mentholysis) of certain molds and bacteria. Since menthol constitutes 50% of the components of mint oil, menthol at the rate of as much as 100 pounds per acre was applied to contaminated soil but did not effect, significantly, any control of the clubroot organism. It would seem that the possibility of the control being the result of menthol leaching into the soil, is remote.

The incomplete sterilization of the soil by the mint plant is indicated by the fact that the ammonifying and nitrifying bacteria were not inhibited as shown by as great a production of nitrates and ammonia in soils having been cropped to mint for three years as in soils which had been cropped to cabbage or corn for a similar length of time. This also tends to eliminate the possibility of decrease in availability of nitrogen being the cause

for depressed yields of other crops following peppermint.

From the foregoing discussion, it appears that the mechanism by which the peppermint plant controls the clubroot organism is not a simple, direct effect. It is therefore theorized that it may be a somewhat complex antibiotic, plant-soil-microorganism relationship, which may take place in the following manner: unidentified excretions from the living mint plant, or products of decomposition of parts of the mint plant (perhaps the stolons) are either stimulating to certain microorganisms which in turn produce an antibiotic that kills the clubroot organism, or the products of the plant are inhibitory toward certain organisms allowing rapid increase of the uninhibited organisms, the latter then producing an antibiotic that kills P. brassicae. Further, the basis for this assumption is found in a preliminary experiment carried out in the greenhouse in the following manner: live mint stolons and the dead mint hay which covered them in the field were collected from the surface of the soil, taken to the greenhouse and packed into two-gallon pots and covered with water. This medium became covered with a fungus within four days and the liquid developed a peculiar odor. Soil contaminated with clubroot was then saturated with this inoculum. As the soil dried, a mycelium covered it and then disappeared leaving it with an entirely different odor in contrast to the untreated soil. Cabbage

planted in these soils after 10 days were healthy and only one plant in twenty developed any infection from clubroot. Both fungi and bacteria were isolated from the liquid on the mint stolons and an attempt is being made to reconstruct the mechanism of control, in vitro.

### SUMMARY

The clubroot organism, P. brassicae, has been controlled in contaminated muck soil by means of a rotation in which peppermint, M. piperita, was grown.

Field and greenhouse experiments have shown that the disease is greatly reduced on a soil after only one year of cropping to peppermint.

The yield of marketable cabbage was increased from 6.6 tons per acre when grown on contaminated soil, to 23.5 tons per acre after a crop of peppermint.

The infection of cabbage plants by clubroot on soil cropped to peppermint for three years was only 4 percent as compared to 100 percent on the plots continuously cropped to cabbage.

The rapidity with which the organism disappeared from the soil, indicated that the mechanism is not one simply of the elimination of the host plant.

Further experimentation under controlled conditions indicates that the mint plant is responsible for the production of an antibiotic or a chain of antibiotics which are toxic to the clubroot organism.



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# APPENDIX I

Plan, data, and analysis of variance for split plot design with a dummy treatment

Field plots harvested July 20, 1949  
Walkerton, Indiana

A. Plot plan and data. Percentage of cabbage plants infected with clubroot. Left half of plots with 0 and 1 year of mint had been treated with extra mint plant material in 1948. The treatment, 2 years of mint, was still in mint in 1948 and did not receive the additional treatment. In the calculations the latter is the dummy treatment.

|                     | Replication 1 |       |      | Replication 2 |      |      | Replication 3 |       |      | Replication 4 |      |      |
|---------------------|---------------|-------|------|---------------|------|------|---------------|-------|------|---------------|------|------|
| Plot No.            | 1             |       |      | 5             |      |      | 9             |       |      | 13            |      |      |
| No. of yrs. of mint |               | 2     |      |               | 0    |      |               |       |      |               | 1    |      |
| Percent infection   | 11.9          |       | 4.3  | 87.9          |      | 96.1 |               |       |      | 0             |      | 0    |
| Sum of 2 subplots   |               | 16.2  |      |               | 184  |      |               |       |      |               | 0    |      |
| Average             |               | 8.1   |      |               | 92   |      |               |       |      |               | 0    |      |
| Plot No.            | 2             |       |      | 6             |      |      | 10            |       |      | 14            |      |      |
| No. of yrs. of mint |               | 0     |      |               | 2    |      |               | 1     |      |               |      |      |
| Percent infection   | 62.5          |       | 89.7 | 6.3           |      | 18.6 | 54.5          |       | 57.9 |               |      |      |
| Sum of 2 subplots   |               | 152.2 |      |               | 24.9 |      |               | 112.4 |      |               |      |      |
| Average             |               | 76.1  |      |               | 12.5 |      |               | 56.2  |      |               |      |      |
| Plot No.            | 3             |       |      | 7             |      |      | 11            |       |      | 15            |      |      |
| No. of yrs. of mint |               | 1     |      |               |      |      |               | 2     |      |               | 0    |      |
| Percent infection   | 0.0           |       | 7.1  |               |      |      | 44.2          |       | 17.2 | 26.8          |      | 19.3 |
| Sum of 2 subplots   |               | 7.1   |      |               |      |      |               | 61.4  |      |               | 46.1 |      |
| Average             |               | 3.6   |      |               |      |      |               | 30.7  |      |               | 23.1 |      |
| Plot No.            | 4             |       |      | 8             |      |      | 12            |       |      | 16            |      |      |
| No. of yrs. of mint |               |       |      |               | 1    |      |               | 0     |      |               | 2    |      |
| Percent infection   |               |       |      | 0.0           |      | 2.3  | 86.7          |       | 86.3 | 18.8          |      | 0.0  |
| Sum of 2 subplots   |               |       |      |               | 2.3  |      |               | 173   |      |               | 18.8 |      |
| Average             |               |       |      |               | 1.2  |      |               | 86.5  |      |               | 9.4  |      |

B. Effect of number of years of mint.

| Years of mint | Percent Infection |         |
|---------------|-------------------|---------|
|               | Totals            | Average |
| 0             | 555.3             | 69.4    |
| 1             | 121.8             | 15.2    |
| 2             | 121.3             | 15.2    |
| Sum           | 798.4             |         |
| Average       | 33.3              |         |

Effect of (1) number of years of mint

(2) additional mint plant material which was chopped and spread on half of each plot

(3) their interaction

| Extra mint plants added | Years of mint |       | Sum   | Average |
|-------------------------|---------------|-------|-------|---------|
|                         | 0             | 1     |       |         |
| 0                       | 263.9         | 54.5  | 318.4 | 39.8    |
| Some                    | 291.4         | 67.3  | 358.7 | 44.8    |
| Sum                     | 555.3         | 121.8 | 677.1 |         |
| Average                 | 69.4          | 15.2  | 42.3  |         |

C. Analysis of variance

| Source of variation                     | df | Sum of square | Mean square | F     | F     |       |
|-----------------------------------------|----|---------------|-------------|-------|-------|-------|
|                                         |    |               |             |       | 1%    | 5%    |
| Analysis for all 3 main plot treatments |    |               |             |       |       |       |
| Total sum of squares                    | 23 | 27936.19      |             |       |       |       |
| Main plots S.S.                         | 11 | 26825.07      |             |       |       |       |
| Replications S.S.                       | 3  | 6754.55       | 2251.52     | 3.08  | 9.78  | 4.76  |
| Treatments S.S.                         | 2  | 15678.27      | 7839.14     | 10.71 | 10.92 | *5.14 |
| Error "a"                               | 6  | 4392.25       | 732.04      |       |       |       |
| Subplot total                           | 12 | 1111.12       |             |       |       |       |

Analysis for 2 main plot treatments, subplot treatments, and their interaction

|                                      |    |          |          |       |         |      |
|--------------------------------------|----|----------|----------|-------|---------|------|
| Between 0 and 1 year<br>of mint S.S. | 1  | 11745.14 | 11745.14 | 16.04 | **13.74 | 5.99 |
| Error "a"                            | 6  |          | 732.04   |       |         |      |
| Extra mint S.S.                      | 1  | 101.50   | 101.50   | 1.02  | 10.04   | 4.96 |
| Extra mint x years S.S.              | 1  | 13.51    | 13.51    | .14   | 10.04   | 4.96 |
| Error "b"                            | 10 | 996.11   | 99.61    |       |         |      |