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TESTING CABBAGE PLANTS FOR CLUBROOT RESISTANCE

(*PLASMODIOPHORA BRASSICAE* WORON.)

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1. In testing cabbage plants for resistance to clubroot the field method has been replaced by two more rapid and more reliable methods, respectively indicated as the soil inoculation method and the dipping method.

2. Using the soil inoculation method, testing is done in artificially infected soil in the glasshouse. This soil should be slightly acid, adequately moist and sufficiently warm (26 °C). The required inoculation of the soil is obtained by mixing 1 part of infected roots with 25 parts of soil. A stronger dilution of the inoculation material is not desirable.

3. With the dipping method the young plants are dipped for some time into a suspension of water and infected soil, after which the plants are set out in a healthy soil. Although dipping for 1 minute can be sufficient, it is safer to dip the plants for some hours. A suspension of 1 part of infected roots and 5 parts of soil to 2 parts of water is sufficiently infective. The actual infection takes place after the dipped plants have been transplanted. To become thoroughly infected, they have to remain in the soil for at least 2 to 3 weeks, depending partly on the pH and the temperature of the soil, as indicated under 2.

4. Five to six weeks after the beginning of the tests normal susceptible plants are heavily infected.

INTRODUCTION

Clubroot is a very common disease in Crucifers and is caused by the fungus *Plasmodiophora brassicae* WORON. The roots of infected plants become swollen and form irregular galls, which greatly reduce normal root activity (fig. 1). The plants lag behind in development and sometimes show symptoms of wilting. This disease can cause considerable damage.

On badly infested soil the cultivation of susceptible crops is generally not justified. Such a soil remains infested for several years because the rest spores of the fungus long remain germinable. To control this soil parasite, sublimate and calomel solutions are used. In concentrations required to control the disease completely these means are also detrimental to the plants (1). The control is preventive, as these fungicides are applied at planting time. As soon as disease symptoms have appeared, no measures can be taken.

The above difficulties can be prevented by using resistant varieties. Since 1952 investigations on this problem have been carried out by our Institute.

Inoculation methods

a. Field method

With the field method, which had been used during the first few years, sowing was done on a seed bed in the spring. After 5 to 6 weeks the plants were transferred to a badly infested commercial cabbage field. They were examined in the autumn. This method of testing was not very reliable, and in order to get better results, it was replaced by the following two procedures.

b. Soil inoculation method

The testing of the young plants grown on in the seed bed is done in artificially infected soil in the greenhouse. The soil is infected by mixing it with diseased roots that have been cut up fine. The soil temperature is from 20 to 25 °C, and the soil is kept sufficiently moist. In this way severe clubbing is obtained after 5 to 6 weeks.

c. Dipping method

The roots of the young plants are dipped for a short time into a suspension of water and infected soil (fig. 2) after which the plants are set out in a healthy soil. This method, too, has produced good results.

For each of the two methods the effect of some environmental factors on the occurrence of infection has been determined. The trials were usually planted in two replications, with 20 to 50 plants per number, using the susceptible variety *Langedijker Early Red* as a test variety. Use was made of affected root material from a field at Bobeldijk (North-Holland). In addition to the percentage of diseased plants the degree of clubbing was determined on a scale ranging from 1 (slightly affected) to 8 (badly affected). The following tables give average values, while the degrees of clubbing only relate to the diseased plants.

Soil inoculation method

Effect of degree of infection of the soil and pH

Some separate trials showed that when the infection material (affected roots) was diluted from 1/9 to 1/288 all plants were clubbed after 24 days, and that only 38 % of the plants were slightly clubbed after 47 days at a dilution of 1/150,000. Apparently a strong dilution can cause some amount of clubbing, but no complete infection.

It was also found that when the degree of acidity of the soil in the range of pH = 5 à 6 to pH = 8 (pH – H_2O) was varied, clubbing was reduced as the pH became higher.

In another trial, which will be discussed here more fully, both the degree of infection of the soil and its pH were varied. Use was made of a soil mixture of leaf mould and peat dust with pH = 5.7 (pH – H_2O). By liming the soil the pH was increased to 6.4 and 7.8. Variation in the degree of infection of the soil was obtained by

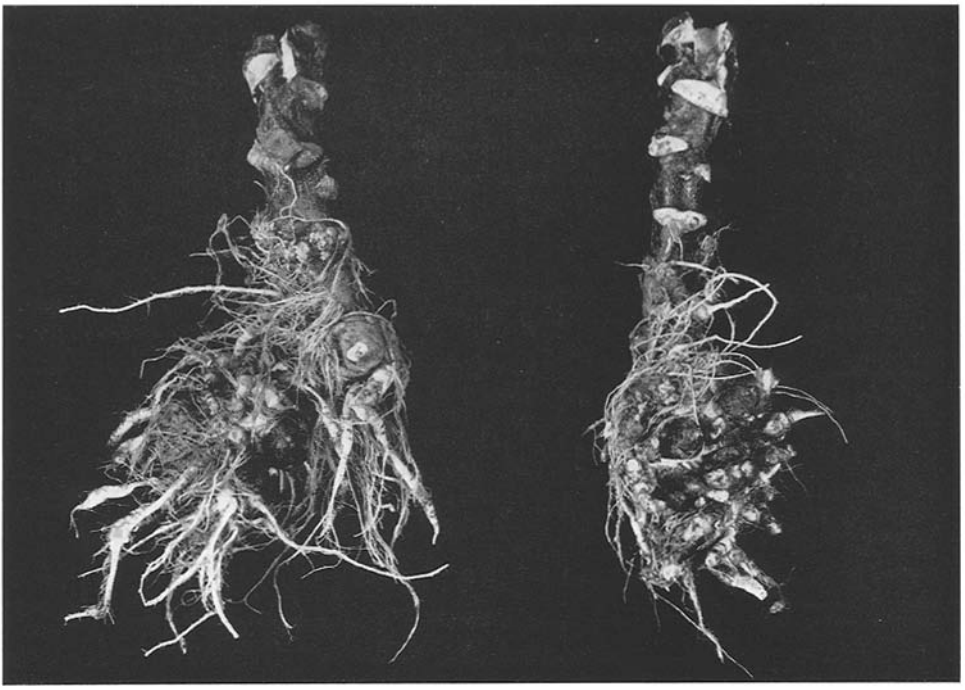

FIG. 1. HEAVILY INFECTED ROOTS

FIG. 2. PLANTS DIPPED INTO THE INOCULATION SUSPENSION

mixing 1 part of affected roots with a varying number of soil parts. Thus dilutions were obtained of 1/25, 1/250, 1/2,500 and 1/25,000. The percentages of diseased plants are represented graphically in fig. 3, and are based on observations made 19 and 43 days after transplanting into infected soil in the greenhouse.

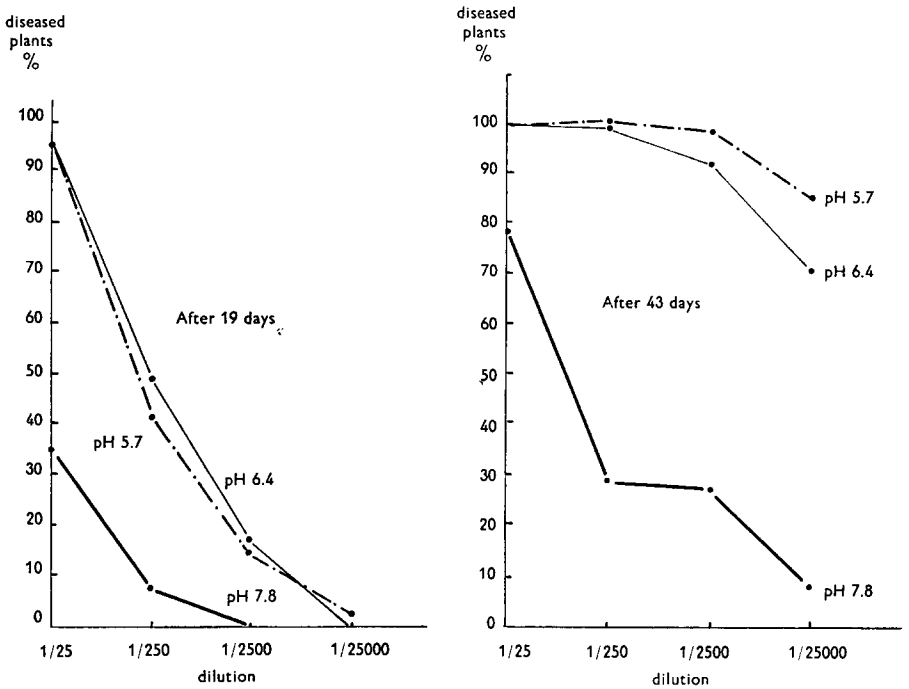

FIG. 3. THE INFLUENCE OF THE DEGREE OF INOCULATION OF THE SOIL AT 3PH'S ON THE PERCENTAGE DISEASED PLANTS

The higher the degree of infection of the soil and the lower the pH, the greater is the amount of clubbing. After 19 days a high degree of infection was obtained only at pH 5.7 and 6.4 at the strongest concentration of the inoculation material, namely 98% diseased plants. Consequently, to prevent susceptible plants being mistaken for resistant ones, 19 days must have been too short a period in this case. After 43 days all plants were clubbed and in addition the mean degree of infection of the diseased plants had increased.

At a pH of 7.8 an exposure period of 43 days, even at the strongest concentration of the inoculation material (dilution 1/25), was not sufficient to obtain 100% clubbing. If all plants are to be diseased after 43 days, it is possible to use a little less inoculation material (dilution 1/250) only when the pH is about 6 (5.7 and 6.4). At a dilution of 1/2,500, even at a low pH, all the susceptible plants were not yet diseased after 43 days.

Effect of soil moisture

As incidental observations seemed to indicate that infection makes slower progress and reaches less severe proportions in a dry than in a moist soil, a series of trials was initiated to obtain further information on this point.

The infected soil was either left continuously dry or kept continuously moist. In some cases further variation was obtained by making the soil thoroughly moist for 1 or 2 days after setting out the plants, and then leaving it dry, or by keeping the soil very moist. Observations were made after 2, 3 and 4 weeks. For comparison of the results of the various trials the number of diseased plants and the degree of clubbing are given as index numbers, counting the results from the moist soil as 100 (see table 2).

Comparison of the index numbers for dry and for moist soil shows that in all cases the percentage of diseased plants on dry soil was lower, and that in 5 of the 7 cases the degree of clubbing was also lower.

Moistening the soil for 1 or 2 days after transplanting was not sufficient; in 2 of the 3 cases a constant excess of moisture was not sufficient either.

DIPPING METHOD

Effect of dipping period

The time during which the plants remained in the suspension (the dipping period) was varied from 1 minute to 24 hours, in a series of 3 experiments. As the results of the 3 experiments all pointed in the same direction they are represented jointly in table 2 as average index numbers. To determine these numbers, the results from a dipping period of 24 hours were counted as 100, except in one case where only 2 dipping periods (1 minute and 7 hours) were used and the results from 7 hours dipping were counted as 100.

TABLE 1. NUMBER OF DISEASED PLANTS AND DEGREE OF CLUBBING, REPRESENTED AS INDEX NUMBERS, (24 HOURS = 100) AFTER DIFFERENT DIPPING PERIODS. OBSERVATIONS WERE MADE AFTER THE DIPPED PLANTS HAD BEEN KEPT IN A HEALTHY SOIL FOR 3 TO $4^1/_2$ WEEKS

	Dipping period						
	1 min.	2 hours	4 hours	7 hours	8 hours	14 hours	24 hours
Number of diseased plants . .	100	100	100	100	103	103	**100**
Degree of clubbing	107	110	100	93	113	93	**100**

Table 1 shows that dipping for 1 minute produced the same results as dipping for 24 hours. In most cases all plants were clubbed after 3 to $4\frac{1}{2}$ weeks. In one experiment a few plants showed no clubbing, but this was in no way connected with the dipping period applied.

These results show that dipping for 1 minute should be sufficient. However, in a few cases a short dipping period produced less clubbing, so we prefer a dipping period of some hours.

When the plants after being dipped for a short time were cleaned thoroughly before planting, they did not become infected. Evidently, infection is not brought about during dipping, but is afterwards caused by spores which, in planting, are carried by inoculation suspension adhering to the roots.

TABLE 2 NUMBER OF DISEASED PLANTS AND DEGREE OF CLUBBING AT DIFFERENT LEVELS OF SOIL MOISTURE SHOWN BY INDEX NUMBERS. THE RESULTS FROM CONTINUOUSLY MOIST SOIL ARE COUNTED AS 100

Observed after	Dilution inoculation material	pH of soil	Soil dry		Moist 1 day		Moist 2 days		Continuously moist		Continuously very moist	
			clubbing		clubbing		clubbing		clubbing *)		clubbing	
			number	degree	%	degree	number	degree	number	degree	number	degree
2 weeks	1/100	6.9	66	73	89	67	117	87	100 (64)	100 (3.8)	–	–
		5 à 6	71	68	–	–	–	–	100 (49)	100 (2.5)	86	84
3 weeks	1/100	6.9	82	63	96	76	82	85	100 (100)	100 (5.9)	–	–
		5 à 6	82	85	–	–	–	–	100 (92)	100 (3.4)	93	85
4 weeks	1/100	6.9	90	99	84	101	96	96	100 (100)	100 (7.5)	–	–
		5 à 6	82	106	–	–	–	–	100 (89)	100 (3.7)	100	122
	1/25	±5	94	88	92	100	–	–	100 (100)	100 (5.7)	–	–

*) Between brackets: the actual percentages of diseased plants and degree of clubbing.

Dilution of the dipping suspension

In most experiments a soil mixture was used consisting of 1 part of affected roots and 5 parts of soil, from which the dipping suspension had to be prepared. To this end the soil mixture was diluted with water as greatly as 1:2. The results from using dilutions of 1:20 and 1:200 were ascertained in a special trial. The plants were dipped at 17 °C and 26 °C for 2 or 24 hours. After 28 days the degree of clubbing was determined (see table 3).

TABLE 3. EFFECT OF DILUTING THE DIPPING SUSPENSION ON INFECTION, AT 2 TEMPERATURES AND 2 DIPPING PERIODS

Dipping temperature	Dipping period in hours	Percentages of diseased plants at the dilutions			Degree of clubbing at the dilutions		
		1 to 2	1 to 20	1 to 200	1 to 2	1 to 20	1 to 200
26°C	2	100	96	80	6.9	5.9	5.0
	24	100	96	81	6.2	5.4	4.7
	av.	100	96	81	6.6	5.7	4.9
17°C	2	100	86	71	6.4	4.6	3.7
	24	100	100	92	5.9	5.6	3.5
	av.	100	93	82	6.2	5.1	3.6

The dilution of the inoculation material had a large effect. After 28 days only a dilution of 1 part of soil mixture with 2 parts of water produced infection of all susceptible plants. The degree of infection is also smaller at the stronger dilutions.

The dipping temperature has not exerted a clear effect. From experiments (mentioned later in this paper) on the effect of temperature during the dipping period it could be concluded that there is no such effect. The dipping period had no effect either.

The degree of acidity of the dipping suspension

Four degrees of acidity of the dipping suspension (pH–H_2O = 5.0, 5.7, 6.7 or 7.6) have been compared at 17° and 30 °C. The dipping period was 7 hours. The percentage of diseased plants showed no differences and was in all cases 100% after 34 days. The degree of clubbing was between 6.3 and 7.2; at pH = 7.6 it was somewhat lower: 5.7 and 5.8. Apparently, after transplanting, the pH of the suspension carried on the roots soon becomes the same as that of the surrounding soil. At the highest pH this may take a little more time. So we may say that during the inoculation period it is the pH of the soil in which the plants are transplanted which determines the degree of clubbing. In the present experiments the pH was 5.5.

The exposure period

As we have seen above, infection does not take place during dipping but after transplanting into healthy soil by means of the spores carried on the roots.

In order to ascertain how long the plants, after transplanting, have to be exposed to become diseased, 2 experiments were made in which the dipped plants were kept in the soil for 2 to 20 days. After digging out, and washing the roots thoroughly, the

plants were transferred to healthy soil. After some time the percentage of diseased plants and the degree of clubbing were determined.

No differences in the degree of clubbing were observed. The different percentages of diseased plants are shown in table 4.

TABLE 4. EFFECT OF THE EXPOSURE PERIOD ON THE PERCENTAGE OF DISEASED PLANTS

Year	Number of days between dipping and observation	Exposure period in days					
		2	4	8	12	13	20
1958	36	–	91	91	–	100	–
1959	53	24	57	87	91	–	100

The table shows that the exposure period should be at least 2 weeks. Longer periods are likely to produce more reliable results.

When the roots after being exposed for 7 hours were washed, no symptoms were observed.

The temperature during the exposure period

The temperature during dipping has little effect on infection, as was shown by experiments in which the dipping temperature varied from 1 to 30 °C and dipping periods of 2 and 24 hours were used (See also table 3). This agrees with the conclusion that infection does not occur during the dipping period.

To determine the effect of temperature after transplanting, 2 experiments were made in which plants were placed in the dipping suspension for 7 hours, and then set out in peat dust for 10 days at 6, 14, 20, 23 or 26 °C.

After washing them thoroughly they were transplanted into a healthy soil (pH = 5). Clubbing was determined 38 or 43 days after dipping. The results are shown in fig. 4.

In the range of temperatures studied the susceptible plants were infected most rapidly at 26 °C; also the degree of clubbing was highest at this temperature. It should be noted that when the roots were being washed all plants at 26 °C already showed symptoms of clubbing, so after being exposed for 10 days.

EFFECT OF GROWTH OF THE PLANTS ON THE APPEARANCE OF CLUBROOT SYMPTOMS

The typical clubroot symptoms result from reactions of the roots to infection by spores. Under conditions that are favourable for plant growth these reactions will be strongest. Frequently, however, the symptoms are less marked. Thus plants growing in a soil with a bad structure often produce less clear symptoms than plants in a soil of good structure. In one experiment the amount of clubbing was less at a low pH than at a somewhat higher pH, at which the plants made better growth. After infection has taken place the pH has little effect. In very moist soils root development is sometimes unsatisfactory, and the degree of clubbing is then also lower. In a very dry soil the slime mould is not very active. Sometimes root growth is also reduced, and this further inhibits the appearance of symptoms. Root growth is also reduced by attack of root parasites, e.g. *Phoma lingam*. This may be the reason why plants attacked by them often show no symptoms.

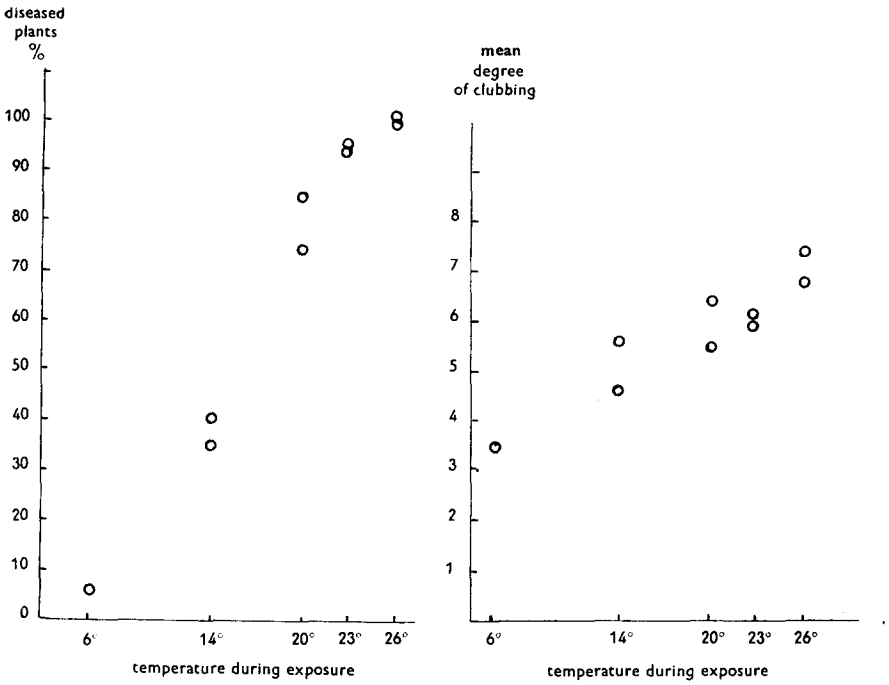

FIG. 4. THE INFLUENCE OF TEMPERATURE DURING AN EXPOSURE PERIOD OF 10 DAYS ON THE PERCENTAGE DISEASED PLANTS AND THE DEGREE OF CLUBBING

On very young plants, of which the roots are still small, the symptoms of clubbing may be less pronounced under unfavourable conditions. From a number of experiments, with plants from 20 to 68 days old, it was shown, however, that the age of the plants generally does not play a part. Both old and young plants can be readily infected.

DISCUSSION

The testing of cabbage plants for resistance to clubroot is best done by the soil inoculation and the dipping method. The field method produces less satisfactory results. With the first two methods, just as with the field method, infection takes place while the plants are in the soil, which should be slightly acid and sufficiently moist. The results of the above experiments agree with those of WELLMAN (7), LARSON and WALKER (4) and COLHOUN (3).

At a high pH the germination of the rest spores is inhibited. Furthermore germination requires a certain amount of moisture, and the movement of the naked swarm spores is only possible in water. In our experiments clubbing was most severe at 26 °C. MONTEITH (6) and WELLMAN (7) found 24° or 25 °C to be the optimal temperature. According to BREMER (2), COLHOUN (3) and MACFARLANE (5), the degree of infection has a clear influence.

However, the effect of the spore concentration appeared to be dependent on the experimental conditions. This was also the case in our trials.

The conditions most favourable for carrying out resistance tests can be described as follows. Using the soil inoculation method the required degree of infection of the soil can be achieved by mixing 1 part of infected roots with 25 parts of soil. After transplanting the young plants the soil should be kept adequately moist for the first 3 weeks, at a temperature of 26 °C. Moreover, it should have a good structure with pH not higher than 6. After three weeks the soil is kept drier and the temperature is lowered to 17 to 20 °C, to prevent damage by other fungi as much as possible. Although some plants may already show symptoms after 10 days, severe clubbing of all the susceptible plants occurs after 5 to 6 weeks.

In practice the pH and moisture requirements can be easily met. By mixing peat dust through the soil the pH can frequently be lowered; in many cases this has a favourable effect on soil structure. In pure peat dust the growth of cabbage plants was sometimes slower, so that the symptoms were less pronounced. The maintenance of a temperature of 26 °C requires special provisions. When testing at lower temperatures it will be longer before all the susceptible plants are infected. This can be checked by including a susceptible control. When the temperature falls below 17 °C infection of the susceptible plants may be incomplete. In the summer months testing can be done outside, in the other months this has to be done in a greenhouse with bottom heating.

The plants to be used for testing may be 3 to 4 weeks old. At the end of the testing period these plants will be 8 to 10 weeks old. Despite their fairly large size, the resistant specimens are still fit for transplanting into a commercial cabbage field to judge them for other characters. The plants to be tested could also be sown out directly into infected soil. In that case there may be some damage by fungi.

Using the dipping method the plants are placed for 2 hours in a suspension consisting of 1 part of infected soil and 2 parts of water. The infected soil is made up of 1 part of affected roots and 5 parts of peat dust or garden soil. The temperature during dipping has no effect. Then the plants are transplanted into healthy soil. Although the inoculation suspension carried on the roots remains moist for some days it will be wise to keep also this soil adequately moist for some time. The temperature requirements are the same as for the first method. To obtain the highest degree of infection, the pH of the soil of the seedbed, of the suspension, and of the soil into which the plants are transplanted, should not be too high. Also with the dipping method the plants show clear symptoms after 5 to 6 weeks.

Using the soil inoculation method, much inoculation material is necessary to obtain a heavily infected soil. This soil can be used for several years. In some soils the fungus seems to lose its activity fairly soon. The infected soil used for testing in a greenhouse must always be reserved for this purpose. This is not necessary when the dipping method is applied. Moreover, far less inoculation material will then be needed. This material is kept outside in open containers. Testing for various physiological races is best done by the dipping method. Various races can be mixed just prior to testing, after which a suspension can be made. After being used this suspension can be thrown away. Using the first method, simultaneous testing for various physiological races could be achieved by mixing them all through the soil. After some time changes may occur, for instance through antagonisms between these races, which gives a wrong picture of the resistance. In that case use would have to be made of soil that has just been inoculated. This soil can only be used once, which is great disadvantage.

Both methods are also suitable for the testing of turnips. The plants should not be older than 20 to 25 days. Older plants are not so easily transplanted. Tentative experiments have shown that when the dipping method is applied a suspension of 2 parts of infected soil and 1 part of water should be used. Otherwise insufficient spores will adhere to the roots, these being less ramified than cabbage roots.

REFERENCES

1. BOVER, J. R., The action of mercury as soil fungicide. Ann. Appl. Biol. **38** (1951): 334–347.
2. BREMER, H., B. WEHNELT und E. BRANDENBURG, Methoden zur Prüfung von Bekämpfungsmitteln gegen Kohlhernie. Mitt. Biol. Reichsanst. **55** (1937): 61–79.
3. COLHOUN, J., A study of the epidemyology of club-root disease of brassicae. Ann. Appl. Biol. **40** (1953): 262–283.
4. LARSON, R. H. and J. C. WALKER, Soil treatment in relation to clubroot of cabbage. J. Agr. Res. **48** (1934): 749–759.
5. MACFARLANE, I., Variation in *Plasmodiophora brassicae* WORON. Ann. Appl. Biol. **43** (1956): 297–306.
6. MONTEITH, J., Relation of soil temperature and soil moisture to infection by *Plasmodiophora brassicae*. J. Agr. Res. **28** (1924): 549–561.
7. WELLMAN, F. L., Clubroot of crucifers. U.S.D.A. Wash. Bull. **181** (1930): pp. 31.