TECHNIKON NATAL

# A STUDY ON THE USE OF HOMEOPATHIC PREPARATIONS FOR THE CONTROL OF DOWNY MILDEW ON CABBAGE

R. BRAMMER

# A STUDY ON THE USE OF HOMEOPATHIC PREPARATIONS FOR THE CONTROL OF DOWNY MILDEW ON CABBAGE

**Ronél Brammer**

Dissertation submitted in partial compliance with the requirements for the Masters Diploma in Technology in the Department of Homeopathy at Technikon Natal.

Approved for final submission

10 May 1995

**Professor F.H.J. Rijkenberg** **Date**

M. Sc. (Agric) PhD

SUPERVISOR

## ABSTRACT

The purpose of this research was to evaluate the efficacy of a homeopathic remedy prepared from *Peronospora parasitica* in the treatment of downy mildew on cabbage seedlings by measuring the percentage leaf area infected. Four different homeopathic potencies were used. Three fungicidal and a water treatment serving as controls. Special attention was paid to curative and preventative functions.

The results of Trial 1 were statistically nonsignificant due to over-irrigation by an automatic irrigation system.

In Trial 3PC excessive moisture from mist sprayers positioned near by created a problem. No treatment was different to the control, exept for S151 which was significantly better than the control.

The remaining trials were all successful. S151 acted best when applied preventatively, as in Trials P, and preventatively and curatively as in Trials PC, when compared to the control. It was not as effective when applied curatively as in Trials C, when compared to the control.

Optimo was an effective fungicide when applied curatively as in Trials C, but was not as good as S151 when is applied preventatively or regularly.

Phyton 27 was the least effective fungicide. The disease control it provided was not

better than the control.

The various homeopathic potencies clearly stimulated downy mildew on the crop. The only exceptions were 15CH and 30CH treatments used on a curative basis. Here no stimulation occurred as the disease levels were the same as in the control. The 5CH and 9CH potencies always stimulated the disease, all AUDPC levels being significantly worse than the control.

These results were analysed with ANOVA. Means separation was by LSD tests at the 5% level (95% level of confidence).

## UITTREKSEL

Die doel van hierdie navorsing was om die doeltreffendheid van 'n homeopatiese geneesmiddel, voorberei van *Peronospora parasitica* vir die behandeling van donsige skimmel op koolsaailinge, te evalueer deur die persentasie besmette blaaroppervlak te meet. Vier verskillende homeopatiese sterktes is gebruik. Drie swamdoders en 'n behandeling met water was toegedien as kontroles. Spesiale aandag is geskenk aan genesende en voorkomende funksies.

Die resultaat van Proef 1 was statisties onbeduidend weens oorbesproeiing deur die outomatiese besproeiings-stelsel.

Oormatige vog van missproeiers geplaas aan die kant naaste aan Proef 3PC het 'n probleem veroorsaak. Geen behandeling het betekenisvolle verskille getoon nie, behalwe S151 wat aansienlik beter as die kontrole was.

Die oorblywende proewe was almal suksesvol. S151 het in vergelyking met die kontrole die beste gewerk as dit voorkomend toegedien is soos in Proewe P en voorkomend en genesend soos in Proewe PC. Vergeleke met die kontrole was dit nie so effektief as dit genesend, soos in Proewe C, toegedien is nie.

Optimo was 'n doeltreffende swamdoder as dit genesend toegedien is soos in Proewe C, maar nie so goed as wanneer S151 op 'n gereelde basis toegedien is nie.

Phyton 27 is die mins doeltreffende swamdoder. Die siekteweerstand wat dit verskaf het was gelyk aan of swakker as die kontrole.

Die verskillende homeopatiese sterktes het duidelik donsige skimmel op die gewas gestimuleer. Die enigste uitsonderings was 15CH en 30CH behandelings wat op 'n genesende basis gebruik is. Hier het geen stimulering voorgekom nie aangesien die siektevlakke dieselfde as by die kontrole was.

Die 5CH en 9CH sterktes het altyd die siekte gestimuleer met alle AUDPC-vlakke aansienlik erger as die kontrole.

Hierdie resultate is met ANOVA ontleed. Middeleskeiding is met LSD-toetse op die 5%-vlak (95% vertrouevlak) gedoen.

## DECLARATION

I, Ronél Brammer, declare that the research and the results in this dissertation are my own work and have not been presented for any other diploma of another University or Technikon.

Signed:

5 May 1995

The work reported in this dissertation was performed in the Department of Homeopathy, Technikon, Natal, Durban and University of Natal, Pietermaritzburg.

## DEDICATION

To Ralph Brammer for his support, help and patience and his endless enthusiasm in my project.

## ACKNOWLEDGEMENTS

**I wish to convey thanks and appreciation to all those who have helped me through the year with this project. In particular, I would like to thank:**

Prof. Rijkenberg for his assistance.

Mark Laing for his patience, knowledge and advice without whom this project would not have been possible.

Ralph Brammer for his help with setting up the trials and the typing on the computer.

Kevin Reich for his help with statistical evaluation.

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## DEFINITION OF TERMS

**Avogadro's constant:**

According to the laws of chemistry, there is a limit to how many serial dilutions can be made without losing the original substance altogether. This limit is called Avogadro's number ($6{,}023 \times 10^{23}$). It corresponds to a homeopathic potency of 12CH or 24xH (Fraser, 1992).

**Centesimal potency:**

The simplest form of remedy for discussion is the plant. The whole plant gathered fresh in the flowering season is immersed in alcohol to make an alcoholic solution. This solution is called the mother tincture. One drop of mother tincture is placed on ninety-nine drops of lactose, alcohol or water to obtain a centesimal potency, which is succussed 100 times and referred to as 1CH. One drop of 1CH solution into 99 drops of solvent will create a 2CH solution if succussed and so on. The centesimal scale was Hahnemann's scale of dilutions. This potentization results in the preparation wherein the actual quantity of the original substance becomes less and less (Johnson, 1974). Potencies far beyond Avogadro's number have been found to increase in power (Frazer, 1992). These are not simple solutions, but potentizations because the process of vigorous shaking (succussion) takes place after each dilution (Johnson, 1974).

**Downy mildew (*Peronospora parasitica*):**

Downy mildew is a disease caused by a group of oomycetous fungi that belongs to the family Peronosporaceae. It is an obligate parasite of higher plants (Agrios, 1988) with strictly biotrophic requirements (Dixon, 1984). Attack on *Brassica* host seedlings results in the emergence of long white sporangiophores through stomata. Later on they may appear greyish or light brown on the lower or both surfaces of the cotyledons (Agrios, 1988). Cotyledons may then turn yellow and later shrivel and die. The fungus thrives in cool, clammy conditions and at high humidity (Spencer, 1981).

**Foliar disease rating:**

Disease severity is visually assessed with the aid of logarithmic rating scales of percentage leaf area infected (Horsfall & Cowling,1980 cited by Brophy and Laing, 1992).

Also see attached Appendix 1.

**Homeopathy:**

Homeopathy is a therapeutic method which clinically applies the "Law of Similars" and which uses medicinal substances in infinitesimal doses (Jouanny, 1991).

**Isopathy:**

Isopathy is the treatment of a disease by a drug prepared from the same pathogen that caused the disease. In isopathy, potentization reverses the direction of action, resulting in a break of the organic bond between toxin and body protein. The toxin is then released by the body of the host. This method has been extensively used to treat residual problems when a disease has been overcome early in life, problems after vaccinations, toxic factors of the environment (e.g. heavy metals) and to reduce hereditary problems (Rost, 1986).

**Pharmacopoeia:**

It is an authorative reference work containing monographs of medicines and other therapeutic agents.

It supplies specifications of the sources and standards for the strength and purity of base substances and mother tinctures, formulae and methods of preparation of these substances and their derivative potencies, as well as descriptions of processes for the testing of starting material (Frazer, 1992).

**Succussion:**

Succussion involves the shaking of the fluid in which potentization is carried out, between each successive step of dilution (Twentyman, 1979).

Succussion is the most remarkable and characteristic feature of the manufacture of potencies. Potency energy varies with the number of

succussions. Succussion not only ensures perfect mixing but imparts movement to the fluid thereby creating additional internal kinetic energy. Succussion thus influences the whole molecular field by changing the electromagnetic state of the solvent (alcohol and water) and the solute (Boyd, 1941 cited by Nicholson, 1961). The more the substance is succussed and diluted, the greater the therapeutic effect, while simultaneously nullifying the toxic effect (Frazer, 1992).

## INTRODUCTION:

In the experiment carried out by Netien and colleagues (1972), Netien and Graviou (1978 & 1978),cited by Scofield (1984), it was shown that homeopathic potencies of $CuSO_4$ had curative effects on pea seedlings that are partially poisoned with $CuSO_4$. Poisoned seedlings grew better in $CuSO_4$ 15CH than in distilled water. Growth rhythms could be detected in the seedlings when poisoning or treatments with $CuSO_4$ 15CH were undertaken. However, this study is difficult to follow and made no attempt of statistical analysis (Scofield, 1984).

Another similar experiment was carried out by Graviou (1981) on the effects of $CuSO_4$ 15CH on the rootlet growth of *Phaseolus vulgaris* to show the temporal variation in growth capacity. Grains of wheat were placed in $CuSO_4$ solutions before germination, and were then grown in potentised $CuSO_4$ 15CH and in distilled water as a control. It was claimed that within 36 hours the alpha-amylase levels in the grains placed in the $CuSO_4$ had risen to the same levels as the unpoisoned grains. The levels of the seedlings grown in distilled water remained low (Scofield, 1984).

Graviou and Boiron (1971) tested the effects of homeopathic $CuSO_4$ on the alga *Chlorella vulgaris* which was pre-treated with $CuSO_4$ . Graviou and Boiron showed that the poisoned alga treated with potentised $CuSO_4$ yielded an increase in chlorophyll production, respiration and growth when compared to the control group. (An identical paper was published in 1978 by Boiron and Mann. Without statistical data being present, they claimed that the statistics were significant). In 1977 Moss *et al.* failed to confirm the findings of Graviou and Boiron (Scofield, 1984).

In 1974 Flemming scheduled the effect of $CuCl_2$ on the growth of rootlets of cress seedlings. The seedlings were grown in a solution of $10^{-3}$ $g.1^{-1}$ $CuCl_2$ for 7 days. Initially the statistical information was significant but later the experiment seemed to yield insignificant results (Scofield, 1984).

In 1977 Naret and Glaude used $CuSO_4$ 3CH, 4CH, 5CH, 6CH, 15CH and 27CH as part of culture media for *Enterobacter cloacae, Histeria monocytogenes, Streptococcus basis* and *Edwardsiella tarda.* Their results were statistically analyzed and showed that the potencies 6CH, 15CH and 27CH stimulated growth of the bacteria while 3CH inhibited growth. At potencies of 4CH and 5CH, results were variable (Scofield, 1984).

Hahnemann (1821) himself said: "Every disease is based upon some particular morbid derangement in the feelings and functions of the vital force; and these, in the process of homeopathic cure, by administering a medicinal potency chosen exactly in accordance with the similitude of symptoms, a somewhat stronger, similar, artificial morbid affection is implanted upon the vital power deranged by a natural disease; this artificial affection is substituted, as it were, for the weaker similar natural disease, against which the instinctive vital force, now only excited to effort by the drug affection, needs any to direct its increased energy; but owing to its brief duration it will soon be overcome by the vital force, which, liberated first from the natural disease, and finally from the substituted artificial (drug) affection now again finds itself enabled to continue the life of the organism in health."

The potency doctrine (Hahnemann, 1821 cited by Campbell, 1981):

a) It may be claimed that solutions of medicines are subjected to alternate dilution and succussion. A physical or pharmacological effect continues to be detectable at dilutions greater than would be expected on Avogadro's constant.

b) It may be claimed that the above technique causes a progressive increase in activity, so that the greater the dilution the more powerful the effect.

c) It may be claimed that the succussion and dilution reveals new or latent properties in medicines.

A. Campbell (1981) states: "I therefore agree that the potency theory is, at least in principle, a scientific hypothesis, and should be treated as such".

The founder of homeopathy, Samuel Hahnemann, wrote in the 6th edition of "The Organon", paragraph 270 (quoted by Voegeli, 1989): "if this mechanical treatment is properly done, following the percepts given above the result is that the medicinal substance, which in the crude state existed entirely as physical matter... is ultimately completely subtilized by such higher and higher dynamizations and transformed into a medicinal power that is non- physical. It is no longer perceptible to the senses. However, medicated globuli serve as a vehicle for that medicinal power, either dry, but even more so if dissolved in water; in this condition they reveal the medicinal virtues of that invisible power in the body of the sick individual".

Paragraph 273: "On no account... is it necessary, and even for this reason alone is

it not permissable, to apply more than a single, simple medicinal substance at a time when treating a patient".

The following area of research is quite crucial. There is no reliable experimental method by which the presence or absence of homeopathic potency can be demonstrated. This seriously hinders the investigation of the nature of the potency. Every effort should be made to identify a suitable method. This is most likely to be a bioassay which involves whole organisms, and the isopathic principle may offer promising models. Effects on seed germination, enzyme systems or tissue cultures may be worthy of investigation (Ives, 1983).

Potencies of silver nitrate have been repeatedly used in studies on the growth of wheat seedlings (Pelikan & Unker, 1971; Kolisko, 1978; Jones & Jenkins, 1983 cited by Steffen, 1983).

Copper sulphate has been used in various studies on the growth of plant seedlings and bacteria.

A variety of studies have been published where potencies had been applied to growing organisms which had previously been poisoned by toxic levels of the same substance (Boiron & Mann, 1970 cited by Steffen, 1983).

Based on statistical information on prescriptions dispensed, the homeopathic pharmacies most commonly prescribed potencies in the centesimal series of 6, 12 and 30 (Cook, 1984).

The choice of homeopathic remedies is obvious in the use of isopathic preparations in the relevant cases or remedies prescribed for conditions in humans is used under experimental conditions with similar symptoms or aetiology. In many cases the reason for choosing a
particular remedy is neither stated nor obvious (Scofield, 1984).

The work of Koffler (1965) and Wannamaker (1966, 1968) on the effect of potencies of sulphur or boron on the growth of onions has few available statistics but it is claimed that both substances affect the weight and length of plants as well as mineral content. Koffler (1965) carried out a similar trial, comparing seedlings grown in soil treated with *sulphur* potencies to some treated with placebo. Again it was concluded that growth greatly improved (Scofield, 1984).

Some research on the effect of homeopathic remedies on fungi has been conducted. Bernard (1912) found that low concentrations ($10^{-8}$ - $10^{-10}$) of manganese had a stimulatory effect on the development and formation of conidia of *Aspergillus niger* in culture (Scofield, 1984).

Narodetzki (1938) used higher potencies of sodium borate and mercuric chloride and found that some potencies decreased mycelial growth of *A. niger*, and a 18CH inhibited growth (Scofield, 1984).

Another experiment was conducted by using 14 homeopathic remedies in the 20th potency on aflatoxin production and mycelial growth of *Aspergillus parasiticus* in a liquid rice-flour medium. Again some remedies stimulated while others inhibited growth (Scofield, 1984).

Homeopathic trials reported in the plant pathology literature have mainly been conducted by Indian workers. Literature on the control of fungal and viral diseases of plants by homeopathic preparations were published by Khanna and Chandra (1976, 1977 & 1978) cited by Scofield (1984).

The experiment carried out by Khanna and Chandra involved the treatment of fruit rots caused by fungi. By using several homeopathic potencies of different remedies they determined the inhibition of germination of specific fungal spores by using hanging drop preparations.

Certain remedies and specific potencies completely inhibited spore germination, when tested on fruit infected with the fungus either before or after treatment. Most potencies that provided positive results were not those commonly used by homeopathic physicians.

These experiments contributed to homeopathy by developing useful ways of practical application in the field of agriculture. The results are summarized in Table 1.

Table 1. Effect of some homeopathic drugs on the development of fruit rots.

| Remedies in different potencies | | Percentage rot developed | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Fusarium roseum* | | *Pestalotia psidii* | | *Pestalotia mangiferae* | |
| | | Pre-I.T. | Post-I.T. | Pre-I.T. | Post-I.T. | Pre-I.T. | Post-I.T. |
| *Arsenicum album* | 1 | 90.7 | 85.3 | | | 41.3 | 41.5 |
| | 60 | | | 2.7* | 71.8* | | |
| | 65 | | | 7.1* | 56.3* | | |
| | 89 | | | | | 35.2* | 38.0* |
| | 90 | | | | | 32.6* | 42.0 |
| | 181 | | | 0* | 73.5 | | |
| *Kali iodide* | 1 | | | 4.7* | 53.8* | | |
| | 20 | | | 8.4* | 63.8* | | |
| | 24 | | | 21.7* | 45.0* | | |
| | 61 | | | 6.3* | 71.6* | | |
| | 87 | | | 0* | 63.2* | | |
| | 149 | 0* | 0* | | | | |
| *Phosphorus* | 35 | 88.8 | 86.1 | | | | |
| | 50 | | | | | 40.4 | 38.5* |
| *Asvagandh* | 100 | | | | | 34.9* | 39.6* |
| *Lycopodium clavatum* | 190 | | | | | 2.5* | 2.0* |
| *Zincum sulph.* | 1 | | | | | 38.4 | 36.6* |
| | 2 | | | | | 40.0 | 38.9 |
| *Thuja occ.* | 87 | 2.8* | 1.4* | | | | |
| Control | | 91.3 | 87.8 | 89.2 | 85.5 | 41.5 | 41.8 |

* Significantly different from control at 5% level of probability.
Pre-I.T. = Pre-inoculation treatment with drug.
Post-I.T.= Post-inoculation treatment with drug.
c potencies are used. (c potencies are the equivalent of Korsakovian potencies (K)) (Scofield, 1984)

Khanna and Chandra (1977) also used the hanging drop technique to study the effects of various remedies on the inhibition of spore germination of *Alternaria alternata* (leaf blight on wheat). Certain potencies were sprayed on wheat plants 24 hours prior to infection with the fungus. After 20 days it was found that Arsenicum album 199c had a 41% control and Kali iodide 200c had a 59% control on the fungus.

Chaube (1978) cited by Scofield, 1984 also used the hanging drop technique to assess the control of *Helminthosporium oryzae, Fusarium solani* and *Penicillium decumbens*. A 30CH and 200CH of several non-specified remedies were used. Again, some inhibited spore germination whilst others accelerated it. No statistics exist for this trial.

Khurana (1971) cited by Scofield, 1984 tested the effect of homeopathic remedies on the response of host plants to three papaya viruses and cucumber viruses. He concluded that the treatment of infected plants (i.e. cure) was not as effective as prophylactic treatment. Treatment on systemically infected hosts also seemed to be more effective than on local lesion hosts. Prophylactic treatment resulted in reducing the severity of symptoms and in delaying the appearance of ring spot virus of papaya seedlings. No statistics are available.

Shukla and Joshi (1982) tested the inhibition of pathological effects of sugar cane mosaic virus in sorghum. Inhibition was achieved by using certain homeopathic remedies. No statistics are available (Scofield, 1984).

A field trial by which four homeopathic sprays were applied to rye grass was reported. The trial was aimed at establishing a method for field testing of homeopathic sprays. Arbitrarily chosen dose levels were chosen and no effects were recorded. However, a method for testing homeopathic sprays was established.

Table 2. Results of the first cut - 15 May 1990 (Tonnes DM/ha)

| Nitrogen | Homeopathic preparations | | | | | |
|---|---|---|---|---|---|---|
| Kg/ha | Nil | S1 | S2 | K1 | K2 | Avg |
| nil | 3.7 | 3.4 | 3.8 | 3.4 | 4.1 | 3.7 |
| 50 | 6.1 | 6.3 | 5.8 | 5.7 | 6.2 | 6.0 |
| 100 | 7.6 | 7.6 | 8.0 | 8.4 | 7.8 | 7.9 |
| Avg | 5.8 | 5.8 | 5.9 | 5.8 | 6.0 | |

Sprays S1 and S2 were mixtures of numerous homeopathic preparations but for commercial reasons the formulae was not revealed.
K1 was a liquid potency of *sulphur* 6c.
K2 consisted of *sulphur*, *silica* and *carbo veg.*
Low potencies (6c) were used because of the chronic condition.
The sprays were applied at the start of the growing season and after the first cut.

K1 and K2 were made up by adding 0.2ml of each to 2.5 litres deionized water (March application).
S1 and S2 were made up by adding 0.5ml of each sample to 2.5 litres of deionized water (March and June application).
(Steven, 1991)

Table 3. Results of second cut - 2 July 1990 (Tonnes DM/ha)

| Nitrogen | Homeopathic preparations | | | | | |
|---|---|---|---|---|---|---|
| Kg/ha | Nil | S1 | S2 | K1 | K2 | Avg |
| nil | 0.9 | 1.1 | 1.0 | 0.9 | 1.0 | 1.0 |
| 50 | 3.3 | 3.2 | 3.2 | 3.7 | 3.4 | 3.4 |
| 75 | 4.1 | 4.2 | 4.5 | 4.0 | 4.0 | 4.1 |
| Avg | 2.8 | 2.8 | 2.9 | 2.9 | 2.8 | |

(Steven, 1991)

Grass responds markedly to nitrogen application. The response curve to nitrogen fertilizer is well established. The response to added nitrogen could therefore be used as a reference for comparison with the test substance.

The experiment comprised two replicates each containing three levels of nitrogen fertilizers.

There was a marked response to nitrogen supplementation. Statistical analysis (Harper Adams inhouse program), confirmed that the homeopathic preparations did not have any significant effect on yield.

It seems that the potencies and dose levels chosen were too low. Much work still has to be done on establishing correct substances, potencies and dose levels.

Another trial, conducted by McIvor, has reported success in treating fruit trees, including nectarine, plum and peach trees, isopathically using homeopathic dilutions of the fungus causing leaf curl. A fine hole was drilled into the tree trunk about 16cm above ground level and the 6c potency injected under pressure. Statistical

analysis of the results are not available (Steven, 1991).

Induced sensitisation requires an initial stimulus which causes subtle, persistent systemic biochemical changes in the plant. This defence mechanism is effective against diseases caused by fungi, bacteria and viruses, although specific immunoglobulins have not yet been found in plants (Kuc, 1985; Kuc and Rush,1985 cited by Pegg and Ayres, 1987). During this process energy and metabolites are diverted away from the mainstream of metabolites and phytotoxicity is avoided in the absence of infection.

Inducers result in the synthesis of immunity signals which sensitise the host to respond rapidly when infected or injured.

Pegg and Ayres (1987) carried out several trials using downy mildew and found that immunisation is carried out in plants by abiotic and biotic induction. Systemic protection does not confer absolute immunisation against infection, but reduces severity and symptom development of the disease.

Most *oleracea* species of the *Brassica* genus found in their natural habitats are resistant to downy mildew fungi, as opposed to the susceptible commercial *Brassica*'s. Resistance in *B. oleracae* is expressed as the necrosis of cotyledon mesophyll cells which inhibits growth of hyphae in the host (Greenhalgh and

Dickinson, 1975 cited by Spencer, 1981). Allyl-isothiocyanate is associated with this resistance (Greenhalgh and Dickinson, 1976 cited by Spencer, 1981).

In subsequent work Natti, Dickson and Atkin (1967) identified resistance to two races of *P. parasitica*, in 16 out of 230 plant introduction accessions. Disease resistance was related to heavier waxiness of the foliage.

Barnes and Worteens (1968), have also identified sources of mildew resistance in *B. oleracae*. These are used in breeding programmes in commercial seed firms in the United States (Williams, personal communication cited by Spencer, 1981).

Although heterothallism occurs in *P. parasitica*, reliance on major genes may give only temporary resistance to downy mildew in host plants (Spencer, 1981).

Sporangia of downy mildew are sensitive to environmental hazards, but unfortunately the genus *Peronospora* reacts less markedly to practices reducing viability of spores.

Commonly 40-90% of young seedlings are destroyed by downy mildew causing heavy or total losses of crop yields. In less developed countries the disease control depends on environmental factors. Systemic fungicides have proved to be very effective, although downy mildew is still very difficult to control (Agrios, 1988). Effective homeopathic treatment could thus be of great benefit to the production of *Brassica* crops.

The main chemical control of downy mildew involves the treatment by protectant fungicides (broad spectrum). Adequate protection against the fungus under average disease pressure is only achieved if the grower uses the fungicides according to strict schedules, either based on calendar spray plans or forecasting based on weather parameters.

Protectant fungicides therefore have great merit but under severe disease pressure, e.g., rainfall or high humidity, their limitations are exposed, since they are mostly surface fungicides.

Protectant fungicidal treatment does not result in eradication of the pathogen once this has penetrated the host tissue especially due to the biological and epidemiological characteristics of downy mildew (Spencer, 1981), as the protectant fungicide cannot act systemically.

A drawback of systemic fungicides is that they should be used in mixtures with standard protectants to broaden their spectrum and therefore avoid a build up of resistant pathogen strains. Furthermore, with any fungicide applied to an edible crop, a limiting factor is that the level of chemical residues must be acceptable (Spencer, 1981).

Large amounts of chemicals used in the control of fungi affect the cultivator of the crop, the environment and the consumer.

Crop rotation is of great value in combatting downy mildew, since oospores persist in soil or plant debris and perennating mycelia persist on vegetative parts.

In 1897, Freiherr von Tubeuf stated that prevention against the Peronosporaceae genus may also be carried out by burying or burning of diseased and dead parts of host tissue which may contain hibernating spores.

Fungicidal sprays provide the most effective method of controlling *P. parasitica* on *Brassica* seedlings. Protectant fungicides though, can not be relied upon to give adequate protection against pathogens capable of explosive epidemic development when hosts are very susceptible and when inoculum occurs in great quantities (Spencer, 1981).

A series of trials was recently carried out by Brophy and Laing (1992) on the screening of fungicides for the control of downy mildew on container grown cabbage seedlings.

They found that fungicide treatments were dependent on the levels of the disease. The highest level of disease incidence was found in spring. Furthermore cymoxanil plus mancozeb provided the most effective control against downy mildew.

During spring, oxadixyl plus mancozeb, cupric hydroxide and chlorothalonil gave better protection than mancozeb, the control treatment.

In summer cymoxanil plus mancozeb was most effective. Propamocarb was more effective with the addition of mancozeb. Oxadixl and fosetyl-A1 plus mancozeb provided poor control. Metalaxyl, and metalaxyl plus mancozeb had no effect on *P. parasitica* (Brophy & Laing, 1992).

It has been found that attack of *P. parasitica* on seedlings of *Brassica* host, results in initial deterioration of the cotyledons which later may shrivel and die. At such an early stage of growth loss of the cotyledons may prove fatal to the seedling (Spencer, 1981). Fungicidal treatment results in high costs for the cultivator of *Brassica* species which in turn affect the consumer. This further identifies the importance and need of research involving homeopathy on agricultural practices.

From the review of literature it follows that the purpose of this investigation was to evaluate the efficacy of homeopathy in the control of plant pathogens. Very little statistically significant research has been done on the interaction between homeopathic treatment and plant pathologens. There are opportunities to conduct statistically valid trials, using homeopathic remedies on plants and plant pathogen. The key element is to use a stable, well-defined pathosystem.

The words of a leading German homeopathic physician, H. Wapler: "Our literature is our greatest enemy" (Guttentag, 1966 cited by Scofield, 1984), are still as true now as they were then. Homeopathic literature contains many reports of the successful use of remedies in the treatment of disease, but these are of little value to the practising homeopath, since most reports are of non-controlled trials and are therefore of little value in making a scientific assessment of the efficacy of homeopathy.

Therefore, future experiments conducted in a professional systematic and scientific way may greatly contribute to create a positive view of homeopathy. Experiments conducted in the past on homeopathy and the treatment of fungi need to be repeated before the efficacy of the method can be excepted in homeopathy. It seems that in the past only some potencies have yielded positive results. Thus it is essential that the experiments are repeated to
make the practical application of the method acceptable (Scofield, 1984).

Scofield, cited by Steven (1991), has discussed techniques of much research in an extensive critical review of the potential role of homeopathy in agriculture. He concluded that there was little firm evidence to support efficacy of homeopathic potency despite the great deal of investigation, due to poor experimental methodology. Unfor-tunately, there is no agricultural pharmacopoeia and much work remains to be done to find suitable preparations and correct dose levels.

Crucifer downy mildew (*P. parasitica*), being such a common pathogen in cabbage (*B. oleracea*) seedlings was an ideal opportunity for a scientific research involving the therapeutic principles of homeopathy. Furthermore prior research in this field made it possible to use established controls.

Foliar disease ratings were used in order to determine the prophylactic and curative effects of the homeopathic remedy. This was prepared in different potencies from tissues of *P. parasitica* according to the laws as specified in the Homeopathic Pharmacopoeia. The emphasis of this study was on the preventative and curative effects of the homeopathic remedy only and not on the mechanism of action of the disease. Furthermore the study was limited to the treatment of plants only and did not involve human therapeutics. It is assumed that the growth medium and growth containers were free from any pathogenic agents after sterilisation was performed. Furthermore it is assumed that the experimental design dealt with the environmental and random errors in statistical analysis.

## CHAPTER 1: Pilot Trial

The pilot trial was conducted in order to gain experience with the pathosystem, the spraying equipment, rating scales and data analysis.

Three trials were conducted with eight different treatments. One replication of each treatment was conducted.

The trial was set up towards the back of the tunnel. This resulted in too high temperatures and later trials were set up closer to the wet wall. One replication is not sufficient for statistical analysis, so all further trials had three replications.

While assaying the seedlings for yield loss it was found that these results were too

inaccurate to be statistically significant. Seedlings that grew from the seeds planted varied greatly in size, this not being so because of any disease but rather due to variation in plant vigour.

## CHAPTER 2: Trial 1

### 2.1 **Aim**:

To control downy mildew on cabbage seedlings by using homeopathic potentised downy mildew and fungicidal treatment.

### 2.2 **Materials and Methods**:

**Materials**:

Trays: 72 x 24 celled speedling trays

Pretreatment: Plasdip (left to dry for two days)

Medium: Seedling mix composted pine bark (CPB) obtained from Gromed, Cramond. Available from the Plant Pathology Department of University of Natal.

Seeds: 24 seeds per tray (95% germination rate). Therefore approximately 5445 seeds were required for 72 trays. Cultivar: Green Star, Gloria

Inoculum: Infected seedling trays were received from a commercial nursery

Treatments: Homeopathic potencies : 5CH, 9CH, 15CH and 30CH prepared from *P.parasitica* only.

3 Fungicides: Phyton 27 (Copper phthalate), Optimo (Cymoxanil+Mancozeb) and S151 (Dimethomorph).

Spraying: High pressure sprayers: 4 sprayers for the homeopathic potencies. 1 sprayer for the fungicides. 1 sprayer for the control (water). Volume: sprayed to leaf wetness and ended before runoff. Sprayers used: Efekto Polyspray 2.

Irrigation: Automatically operated in the plant pathology tunnel and commenced twice a day at 08H00 and 14H00.

Fertiliser: 3.1.3.(38) @ 1g/l = 100 ppm N. It was injected into the irrigation system.

**Method**:

Seedling trays were sterilised with a sterilant known as Plasdip making sure that the whole surface area was covered. The seedling trays were left to dry for 2 days. Complete drying is essential to prevent any burning of the seedlings by copper components of the sterilant.

Speedling-24 trays were used so that each replication consisted of 24 plants. Three replications of each treatment were planted. 24 individuals per replication were sufficient to facilitate a valid statistical analysis.

The trial included four homeopathic treatment and three fungicidal treatment serving as controls as well as one control which was inoculated with fungus but treated with water only.

Before filling the seedling trays with CPB the medium was thoroughly moistened as water runs off the surface of dry medium without penetrating. After filling the trays a dibbler was used to ensure uniformity in planting depth of about 8mm.

Two seeds were planted per cell to compensate for unsuccessful germination. Once the seeds were sown the trays were watered by using a hand sprayer till drip - through to ensure good germination.

The trays were stacked in the medium room until radicles began to emerge, which took from 36 to 72 hours depending on the prevailing temperature. During this time it was made sure that the trays were watered sufficiently. Germination problems were negligible due to the high quality of the seed.

As soon as the radicles appeared the trays were moved to the tunnel. The trays were laid out on the same table closest to the wet wall so as to reduce the effects of environmental gradients within the testing site.

The seedlings were irrigated twice a day at fixed times and quantities. The irrigation technique ensured uniform dispersion of 3.1.3 (38) fertiliser which was premixed in the water.

On the seventh day the seedlings were thinned out manually. Seven seedlings were transplanted into ungerminated cells. The seedlings were sprayed with treatment for the first time, after the last irrigation to ensure absorption by the seedlings. The seedlings were sprayed to the standard point before leaf wetness. In each case the sprayers were filled with 500ml of treatment.

The fungicides were applied in the following concentrations:

- Optimo : 2g/litre
- Phyton 27 : 1g/litre
- S151 : 1.2g/litre

The fungicides were prepared at the beginning of the trial only and not before each application. This applied to all replications.

The homeopathic treatments were used in the following potencies:

- 5CH (H1)
- 9CH (H2)
- 15CH (H3)
- 30CH (H4)

The homeopathic treatment was applied as follows:

The volume of water used to spray one tray = 5ml. The ratio of 1 drop of homeopathic treatment to 5ml of water was used. Thus 100 drops of substance were used for 500ml water.

Unfortunately this ratio was calculated incorrectly since it contained too little substance. According to the ratio of centesimal doses the concentration should have been as follows:

Since 1CH = 1/100

therefore 1 part substance + 99 parts solvent = 100

thus 5ml substance + 495ml solvent ($H_2O$) = 500ml

Trial P (prevention) and Trial PC (prevention and cure) were sprayed only. At this stage no disease was detected therefore Trial C (cure) was not sprayed.

On the 10th day Trials P and PC were sprayed again at the same time and with the same quantity of water.

On the 12th day the seedlings were inoculated with diseased seedlings purchased at the local nursery. Two diseased seedlings were transplanted into each tray.

On the 15th day Trial C and PC were sprayed with the eight treatments. Note that Trial P was not sprayed since it was applied preventatively only. Spraying took place again once the irrigation was completed.

On the 18th day the disease had spread. Each seedling was rated according to the percentage of leaf area infected. Cotyledons were rated by visually rating their under surface. The two Trials C and PC were sprayed once again. Rating proceeded until the 26th day of the trial, when the controls were 100% infected.

## 2.3 Results

Table 4: Trial 1

| TRIAL | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 1P | 479.8 | 434.2 | 505.4 | 558.2 | 506.1 | 575.9 | 485.2 | 548.3 |
| RANK | 2 | 1 | 4 | 7 | 5 | 8 | 3 | 0 |
| 1C | 569.6 | 648.3 | 567.5 | 561.9 | 568.2 | 554.0 | 403.7 | 514.0 |
| RANK | 6 | 8 | 4 | 7 | 5 | 3 | 1 | 2 |
| 1PC | 464.8 | 539.1 | 510.8 | 516.0 | 618.2 | 553.9 | 583.9 | 579.2 |
| RANK | 1 | 4 | 2 | 3 | 8 | 5 | 7 | 6 |

Trial P: Prevention
Trial C: Cure
Trial PC: Prevention and Cure
5CH: *P. parasitica* prepared in a 5th homeopathic attenuation
9CH: *P. parasitica* prepared in a 9th homeopathic attenuation.
15CH: *P. parasitica* prepared in a 15th homeopathic attenuation.
30CH: *P. parasitica* prepared in a 30th homeopathic attenuation
OPT: Optimo fungicide
PHY: Phyton 27 fungicide
S151: Dimethomorph fungicide
CON: Control (water)

The statistics of Trial 1 were insignificant, thus the results were ranked to show commonality. The results were very inconsistent and even by using ranks, consistency was not achieved.

## 2.4 Discussion

No trends in the ranking were detected in Trial 1. Automatic mist sprayers were in operation every five minutes. Repeated moisture settled on the seedlings of Trial 1, confounding any trends.

## 2.5 Conclusion

No useful results were recorded but this trial served as an excellent training for later trials.

## CHAPTER 3: Trials 2 and 3

### 3.1 Aim:

To control downy mildew on cabbage seedlings by using homeopathic potentised downy mildew and fungicidal treatment.

### 3.2 Materials and Methods:

**Materials:**

All materials as in Trial 1 with the following changes:

| | |
|---|---|
| Medium: | CPB and Coir Peat for improved water retention. |
| Irrigation: | 4 micro jets for manual irrigation |
| Fertiliser: | Fertiliser ( 3.1.3 (38) @ 1g/l ) was applied manually. |

**Methods:**

As for Trial 1 with the following changes:

The seeds were left to germinate in the tunnel until radicles began to emerge at 36 to 48 hours. The seedlings were irrigated until the point of drip-through once a day only, due to the high water retaining capacity of the coir peat.

Fertiliser was applied manually by watering can and every second day thereafter.

The fertiliser was applied at a concentration of 1g/l and at a rate of 6l per 72 trays.

The fungicides were prepared just before each application for optimal activity, by eliminating the possibility of the hydrolysis of chemical compounds in the fungicides. The homeopathic treatment was applied correctly according to the ratio of centesimal doses as explained in Trial 1.

## 3.3 **Results**

Table 6: Trials 2P & 3P

| TRIAL | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 2P | 550.8 | 553.3 | 562.3 | 535.8 | 465.4 | 481.5 | 146.7 | 377.7 |
| Letters | e | de | de | cde | c | cd | a | b |
| 3P | 559.4 | 542.1 | 556.0 | 525.1 | 457.7 | 452.9 | 143.6 | 405.9 |
| Letters | d | d | d | cd | bc | b | a | b |

For 2P, the F Test was significant at the 100% level.
For 3P, the F Test was significant at the 100% level.
Treatments differing by letters at significantly different at the 95% Level of Confidence.

Trial P: Prevention
Trial C: Cure
Trial PC: Prevention and Cure
5CH: *P. parasitica* prepared in a 5th homeopathic attenuation.
9CH: *P. parasitica* prepared in a 9th homeopathic attenuation.
15CH: *P. parasitica* prepared in a 15CH homeopathic attenuation.
30CH: *P. parasitica* prepared in a 30th homeopathic attenuation.
OPT: Optimo fungicide
PHY: Phyton 27 fungicide
S151: Dimethomorph
CON: Control (water)

In Trials 2P and 3P, S151 was consistently the best fungicide. Surprisingly Optimo was equal or worse than the control. Phyton 27 was significantly worse or equal to the control. All homeopathic treatments were significantly worse than the control. Analysis of variance was significant for both Trials 2P and 3P. Clear trends were observable.

Table 7: Trials 2C and 3C

| TRIAL | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CONT |
|---|---|---|---|---|---|---|---|---|
| 2C | 566.3 | 525.5 | 525.5 | 527.6 | 492.5 | 522.0 | 537.4 | 497.4 |
| Letters | cd | d | abc | abc | a | ab | bcd | ab |
| 3C | 562.7 | 556.2 | 506.1 | 505.7 | 472.2 | 503.5 | 515.1 | 482.4 |
| Letters | c | c | ab | ab | a | ab | b | ab |

For 2C, the F Test was significant at the 99.1% level.

For 3C, the F Test was significant at the 99.7% level.

Treatments differing by letters at significantly different at the 95% Level of Confidence.

In Trials 2C and 3C, S151 was significantly worse than the control. Optimo was the best fungicide in both trials. Phyton 27 was equal to the control. The 5CH and 9CH homeopathic potencies were significantly worse than the control and the 15Ch and 30CH were higher or equal to the control.

Fig. 1: Trial 2P & 3P

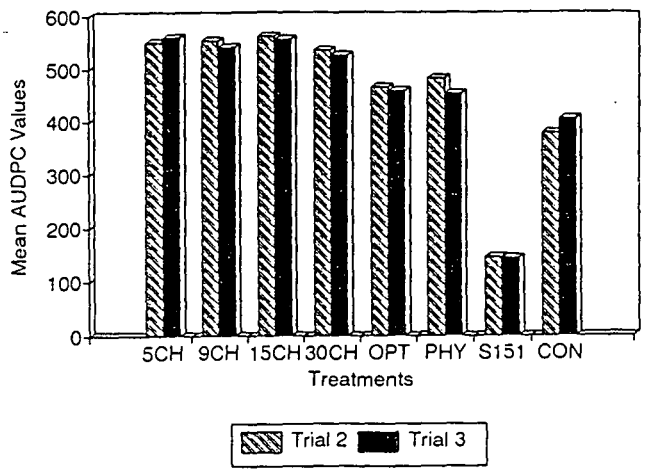


Fig. 2: Trial 2C & 3C

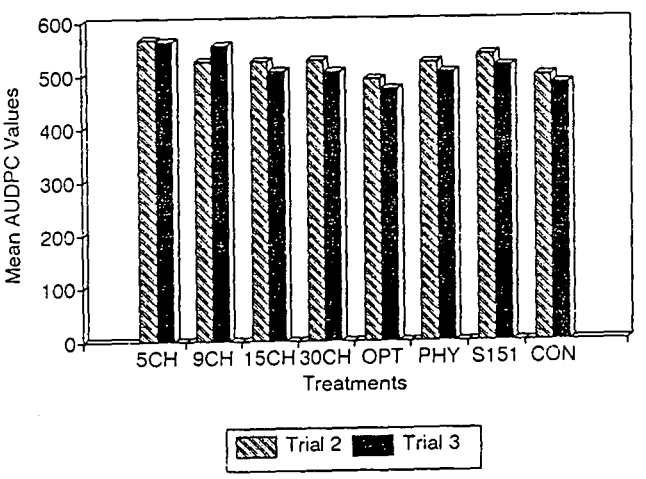


Fig.3: Trial 2PC & 3PC

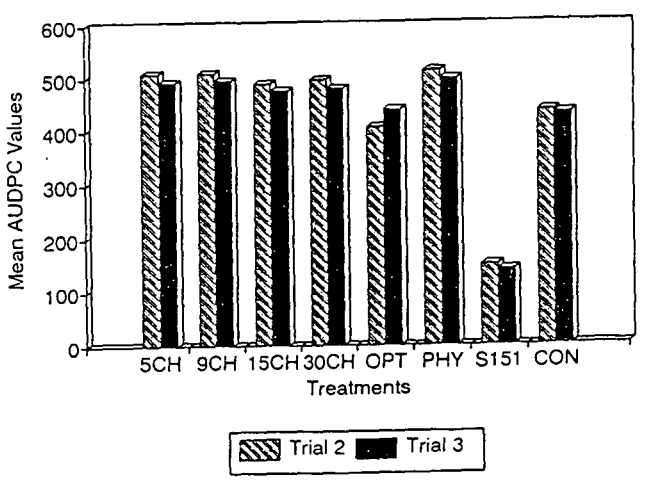

Table 8: Trials 2PC and 3PC

| TRIAL | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CONT |
|---|---|---|---|---|---|---|---|---|
| 2PC | 508.9 | 509.6 | 488.1 | 497.2 | 407.7 | 514.0 | 150.9 | 437.9 |
| Letter | d | d | cd | d | b | d | a | bc |
| 3PC | 492.6 | 495.2 | 475.8 | 479.0 | 439.9 | 497.5 | 140.6 | 432.4 |
| Letter | b | b | b | b | b | b | a | b |

* For 2P, the F Test was significant at the 100% level.
* For 3P, the F Test was significant at the 100% level.
* Treatments differing by letters at significantly different at the 95% Level of Confidence.

In Trials 2PC and 3PC, S151 was clearly the best fungicide. Optimo was significantly equal to the control and Phyton 27 equal or worse to the control. In Trial2 PC most homeopathic treatments were significantly worse than the control whereas in Trial 3 PC they were all equal to the control. However only one significant difference in Trial 2PC and 3PC occured, therefore it was questionable whether a problem existed within the trial.

To get an overview of all significant trial results, the above tables are combined into Table 9.

Table 9: Trials 2 & 3

| TRIAL F sig. Level | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 2P F:0.00 | 550.8 e | 553.3 de | 562.3 de | 535.8 cde | 465.4 c | 481.5 cd | 146.7 a | 377.7 b |
| 2C F:0.009 | 566.3 cd | 525.5 d | 525.5 abc | 527.6 abc | 492.5 a | 522 ab | 537.4 bcd | 497.4 ab |
| 2PC F:0.00 | 508.9 d | 509.6 d | 488.8 cd | 497.2 d | 407.7 b | 514 d | 150.9 a | 437.9 bc |
| 3P F:0.00 | 559.4 d | 542.1 d | 556 d | 525.1 cd | 457.7 bc | 452.9 b | 143.6 a | 405.9 b |
| 3C F:0.007 | 562.7 c | 556.2 c | 506.1 ab | 505.7 ab | 472.2 a | 503.5 ab | 515.1 b | 482.4 ab |
| 3PC F:0.00 | 492.6 b | 495.2 b | 475.8 b | 479 b | 439.9 b | 497.5 b | 140.6 a | 432.4 b |

* Treatments differing by letters at significantly different at the 95% Level of Confidence.

## 3.4 Discussion

### Fungicides

S151 was an outstanding fungicide when compared to the control. When used preventatively and curatively in Trials 2PC and 3PC it yielded excellent results, and even better when applied preventatively in Trials 2P and 3P. However, it did not work as a curative treatment as disease incidence was worse as compared to the control in Trials 2C and 3C.

Note that Trial 3PC showed no significant differences between treatments, except for S151 which showed significant efficacy relative to the control.

Optimo was the best curative fungicidal treatment, as is clear in Trials 2C and 3C where the Optimo results were better or equal to the control. It had little effect in Trials 2P and 3P when used preventatively only, suggesting short activity after uptake in the plant.

Used on a preventative basis, Phyton 27 was worse than the control. On curative application it was again worse than the control, although this result was not statistically significant. For Trials PC it was significantly worse or equal to the control. Overall, it was clearly a poor fungicide for downy mildew control on cabbage seedlings.

All these fungicide results were as expected from prior results, using this and other, parallel pathosystems (Laing, pers. comm.).

**Homeopathic Treatments**

When the homeopathic preparation was applied in a 5CH, plants developed significantly more disease than the control in both Trials 2P and 3P. When the 5CH preparation was applied curatively in Trials 2C and 3C, disease incidence was also significantly worse than the control. In Trial 2PC, application of the 5CH preparation again resulted in a disease spread which was significantly worse than the control. In Trial 3PC it showed no significant difference in effect from the control.

Similarly, the applications of the 9CH attenuations resulted in more disease than the control in Trial 2C, 3C and Trial 2PC.

15CH treatments were also significantly worse than the control in Trials 2P and 3P. However, in Trials 2C and 3C this treatment resulted in disease spread equal to that of the control. In the two PC trials no significant difference showed.

Application of the 30CH potency resulted in significantly higher disease levels than the control in Trial 2P, 3P, 2C, 2PC but were the same as the control in Trials 3C and 3PC.

From the above it is clear that some of the homeopathic treatments had a significant effect in several trials and at several rates. As this research was based on scientific principles these results must be scientifically valid.

Homeopathically they can be explained as follows: the treatments were made up from downy mildew up to a 30th potency. This would mean that after the 12th potency (Avogadro's number of $6.02 \times 10^{23}$), no original substance was left in the solvent (70% alcohol or water). One would ask how is it possible for treatments like the 15th and 30th potencies to have had an effect? According to fundamental research it is said that by the mode of dilution and succusion in an aqueous dilution, for example , each molecule or each ion of the diluted substances is surrounded by water molecules, thus creating a hydrated molecule or ion with new properties. When further dilution is carried out, this molecular arrangement varies not only for each molecule of the hydrated base substance but also in relation to the molecules of the solvent which are not directly related to the substance. It follows that each time the product is diluted in a solvent, a new physio-chemical entity is created, the properties of which are not only proportional to the amount of the original substance used.

The molecules of the diluted substance create a particular arrangement with the molecules of the solvent, resulting in a new physical state. This physical state varies with the height of the dilutions. Each new dilution is marked by the previous physical state. Everything takes place as if the solvent had "memorised" the physical structure of the previous dilution for each dilution. The state may even be perpetuated if there are no longer molecules of the base substance left in the solvent.

Recently Luu-d-Vinh, cited by Jouanny (1991), demonstrated the particular physical state of Hahnemannian dilutions with the Raman laser. By subjecting the dilution to a laser beam (monochromatic light beam) and by recording the diffusions which are produced on the raman spectograph, particular spectra were obtained. These showed that the rays characteristic of the solvent reappear but that they are modified depending on the height of the dilutions and depending on the substance diluted.

This particular physical state is the basis of the reactive and therapeutic potential of a substance diluted in accordance with the Hahnemannian method.

Homeopathy is a therapeutic method which clinically applies the law of similars and which uses medicinal substances in weak or infinitesimal doses.
The fundamental principle is the law of similars which states the parallel action between the toxicological action of a substance and its therapeutic action. These results therefore fit the basic homeopathic theory of Law of Similars.

What is unusual is that the lower potencies stimulated the disease more than the higher potencies. This could have been due to the stimulative property of low potencies which are prescribed in acute, local and physical pathological cases in practice. Higher potencies are prescribed for chronic, deep seated and emotional symptoms.

The results may also suggest that plants react differently to humans on a therapeutic level. This encourages more research in the field of the mechanism of action of homeopathic remedies on plants.

The following questions remain open for further research :

a. When is a plant acutely or chronically infected with a disease ?

b. How much resistance has the plant actually got to cope with the stimulative and curative mechanisms which the homeopathic treatment causes in the plant ?

It may also be important to consider the stage of growth the fungus was in when it was used to make the mother tincture. It could be that different tissues of the fungus would have different effects in the control of the disease.

## 3.5 Conclusion

Trial 3PC also appears to have suffered an experimental problem because no statistical differences between the treatments existed, except the fungicide S151 which was better than the control. This was probably because the distribution of mist sprayers in the tunnel prevented normal progression of the disease.

S151 was a good fungicide when applied preventatively only, as well as preventatively and curatively. It was not a good systemic fungicide since it did not work as a curative application.

Optimo worked well on a curative basis but was not as effective as S151 when this was applied on a more frequent and regular basis.

Phyton 27 was not a good fungicide since the results were always equal or worse than the control.

Only four neutral homeopathic results came up compared to the 16 stimulatory results, with clear statistical significance. The exceptions were when the higher potencies of 15CH and 30CH were used as curative treatment when the disease levels were equal to the control. 5CH and 9CH potencies always stimulated disease. This may be explained by the fact that low potencies in homeopathy always have a stimulatory function especially when given over a longer period of time, as in Trials P and PC.

This strongly suggested that potentised downy mildew had a stimulatory effect on the downy mildew. The 15CH and 30CH potencies lowered the disease incidence when applied over a short period of time. This suggested that higher potencies decreased disease incidence when compared to the lower potencies. This of course makes it economically more viable for the cultivator since it needs to be applied less often in higher attenuations.

Research involving homeopathy on plant pathogens is possible and yields significant statistical results if conducted in a controlled pathosystem.

Therefore, future research conducted in a professional, systematic and scientific way may greatly contribute to create a more positive view of homeopathy. Research on plant pathologens treated homeopathically opens the doors to a new and exciting therapeutic approach in agriculture.

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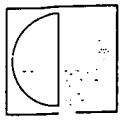
# APPENDICES



# Screening of fungicides for the control of downy mildew on container-grown cabbage seedlings

T. F. Brophy and M. D. Laing*
*Department of Microbiology and Plant Pathology, University of Natal, PO Box 375, Pietermaritzburg, Republic of South Africa, 3200*

**Abstract** Eleven fungicides were screened, over three seasons, for efficacy against crucifer downy mildew, *Peronospora parasitica*, on cabbage seedlings grown in 24-modular polystyrene trays. Starting 10 days after emergence, seedlings were treated with weekly foliar applications of fungicide formulations. Percentage disease severity was assessed weekly using a visual rating scale; the area under the disease progress curve (AUDPC) was calculated for each treatment. A single assessment of final disease severity was an inadequate parameter for comparison of fungicide efficacy. However, AUDPC facilitated clear separation of treatments and provided an integrated measure of disease control. The mixture of cymoxanil and mancozeb was consistently found to be the most effective fungicide against crucifer downy mildew. Other fungicides that provided control included systemics (oxadixyl as a mixture with mancozeb and propamocarb) and protectants (mancozeb, chlorothalonil and cupric hydroxide). All metalaxyl-based fungicides were ineffective.

**Keywords** *Peronospora parasitica*; fungicides; cabbage; seedlings; cymoxanil; oxadixyl; propamocarb; metalaxyl; resistance

## Introduction

Crucifer downy mildew (*Peronospora parasitica* Pers. ex Fr.) is a major foliar pathogen of crucifer seedlings grown in containerized trays in South Africa (Laing, 1984). The lack of any break in time or space (Robinson, 1979) in crucifer seedling production, the explosive epidemic capabilities of downy mildew (Spencer, 1981) and the ideal environmental conditions for the pathogen within the nursery, all contribute to its severity. Control of this pathogen in South Africa is heavily dependent on the use of chemicals.

During the initial years of containerized seedling production (1980 onwards), mancozeb (Dithane M45), chlorothalonil (Bravo), metalaxyl (Ridomil) and metalaxyl plus mancozeb (Ridomil MZ) provided adequate control. However, from 1984, metalaxyl-based fungicides were found to be ineffective (M.D. Laing, unpublished). The existence of metalaxyl-resistant strains of *P. parasitica* was postulated by the authors. Resistance of *P. parasitica* isolates to metalaxyl in other areas has been reported previously (Crute, 1983; Crute and Gordon, 1986). No alternative systemic fungicides for this disease are currently registered in South Africa.

The procedures introduced in this paper provide a rapid, cheap system for the definitive testing of a wide range of systemic and protectant fungicides for efficacy against crucifer downy mildew, for the presence of resistance to metalaxyl, and for the presence of cross-resistance to other fungicides normally active against Oomycetes.

*To whom correspondence should be addressed

0261–2194/92/02/0160–05


## Materials and methods

### Plants

Cabbage seedlings (*Brassica oleracea* L. cv. Gloria Osena, obtained from Starke Ayres, Greytown) were grown in a polyethylene tunnel in 24-module polystyrene trays (Starke Ayres) containing composted pine-bark medium supplied by Starke Ayres. Starting at 7 days after emergence, all seedlings were fertilized three times daily with 3.1.3(38).

### Inoculum

Cabbage seedlings and mature plants infected with downy mildew (*Peronospora parasitica* Pers. ex Fr.) were collected from several commercial seedling nurseries and from farms in the Natal region. Initial inoculation was achieved by shredding fresh plant material and distributing the sporulating infected debris evenly over cabbage seedlings 10 days after emergence; symptoms became apparent after 5–7 days. Subsequent inoculation was achieved by transplanting infected seedlings among healthy seedlings, 10 days after emergence, in a systematic pattern to ensure that all plants received potentially equal amounts of inoculum.

### Fungicides and fungicide application

Eleven fungicide formulations were tested in the initial trial in winter, 1988. Nine of these and two additional mixtures, metalaxyl plus mancozeb and propamocarb plus mancozeb, were retested in spring, 1989. The most successful fungicides from both these trials were further tested in

Table 1. Fungicides, with respective dose rates, tested for efficacy against downy mildew (*P. parasitica*) on cabbage seedlings grown in containerized trays

| Active ingredient | Trade name | Percentage a.i. | Dose 100 litres of product | Supplier in RSA |
|---|---|---|---|---|
| Cymoxanil + mancozeb | Optimo 76 WP | 6 + 70 | 200 g | Agricura |
| Cymoxanil + chlorothalonil | Curzate 680 WP | 6 + 50 | 200 g | Agricura |
| Oxadixyl | Ofurace WP | 8 | 80 ml | Bayer SA |
| Oxadixyl + mancozeb | Recoil WP | 8 + 56 | 330 g | Bayer SA |
| Propamocarb-HCl | Previcur N sl. | 72 | 120 ml | FBC Holdings |
| Propamocarb + mancozeb | Previcur N sl. | 72 | 60 ml | FBC Holdings |
| | + Dithane M45 | 80 | 75 g | Supacryl |
| Metalaxyl | Ridomil WP | 25 | 50 g | Ciba Geigy |
| Metalaxyl + mancozeb | Ridomil MZ WP | 25 + 80 | 50 g | Ciba Geigy |
| Fosetyl-Al + mancozeb | Mikal M WP | 44 + 26 | 350 g | Maybaker/Agrichem |
| Chlorothalonil | Bravo SC | 50 | 100 ml | Shell SA |
| Mancozeb | Dithane M45 WP | 80 | 200 g | Supacryl |
| Copper oxychloride | Cupravit WP | 80 | 400 g | Bayer SA |
| Cupric hydroxide | Kocide WP | 77 | 200 g | Farmers Organisation |

summer, 1990. Fungicides tested and dose rates are given in *Table 1*.

Rates were established on recommendations from the manufacturer or extrapolated from recommendations for control against a similar disease type on another crop. Fungicide suspensions were applied to the seedlings using 'Efekto 2 Litre' pressurized hand-sprayers, until just before run-off (approximately 60 ml per tray). Fungicides were applied 10 days after seedling emergence and then weekly for 4 weeks. The first spray was applied before transplantation of the inoculator seedlings to ensure an even inoculum source for a period of a week. Mancozeb was used in the control treatment in all trials. A water-treated control was not included, in order to reduce interplot interference which would result from increased inoculum pressure from these plots (Van der Plank, 1963). A randomized complete block design with three replications was used for all trials.

### Disease assessment

Disease severity was visually assessed with the aid of logarithmic rating scales of percentage leaf area infected for both cabbage cotyledons and primary leaves (Horsfall and Cowling, 1980), devised by the authors and calibrated on an image analyser. Rating started 7 days after inoculation and was conducted weekly for 4 weeks thereafter. Cotyledons and primary leaves of cabbage seedlings do not develop equal levels of disease: maximum cotyledon area infected can reach 100% whereas primary leaf area infected rarely exceeds 25% (unpublished data). In order to integrate the two components, transformation of the data was necessary. Percentage disease severity (PDS), expressed as a function of cotyledon and primary leaf infection was calculated using the formula $PDS = (C + xP)/2$, where $C$ is the percentage cotyledon area infected with a maximum value of 100%; $x$ is the inverse of the maximum measured percentage primary leaf area infected, and $P$ is the percentage primary leaf area infected of treated plants ($n = 12$). Control efficacy of treatments was also compared using area under disease progress curve (AUDPC) values, computed by trapezoid integration of PDS values plotted against time (Berger, 1988). All results were statistically analysed by one-way analysis of variance (STATSGRAPHICSA, USA).

## Results

Different levels of downy mildew infection were observed during the three trials at different times of the year. In the winter trial, maximum disease severity observed was 12% and no infection of the primary leaves occurred. During spring, infection was most severe with a disease severity of 96% recorded in the least effective treatments. In summer, infection levels were intermediate, 44–66% disease severity. Statistical separation of fungicide treatments was primarily dependent on the prevalent levels of disease; the highest level of significance ($p = 0.01$) was found in spring where the widest distribution in levels of disease occurred.

AUDPC and final PDS (FPDS) values for the winter, spring and summer trials are given in *Tables 2, 3* and *4*, respectively.

Cymoxanil plus mancozeb consistently provided the most effective control against downy mildew. In the spring trial, oxadixyl plus mancozeb, cupric hydroxide and chlorothalonil gave significantly better protection than mancozeb, the control treatment. However, in summer, apart from cymoxanil plus mancozeb, there was no significant difference between any of the fungicides.

Propamocarb did not provide consistent control in all trials; however, the addition of mancozeb did appear to improve its efficacy. Both oxadixyl and fosetyl-Al plus mancozeb provided poor control. Metalaxyl, and metalaxyl plus mancozeb, had no activity against *P. parasitica*. The mancozeb component in the mixture was insufficient to provide any independent control.

## Discussion

The results obtained from this work are consistent with other research findings. A positive interaction effect was found between mixtures of protectant and systemic fungi-

Table 2. Effect of fungicide applied as a foliar spray, during winter 1988, on the disease severity of downy mildew on cabbage seedlings grown in containerized trays

| Treatment | FPDS[a] | Rank | AUDPC[b] | Rank |
|---|---|---|---|---|
| Cymoxanil + mancozeb | 0a[c] | 1 =[c] | 0.0 | 1 |
| Cymoxanil + chlorothalonil | 2a | 1 = | 7.4 | 2 |
| Oxadixyl | 10de | 8 = | 71.4 | 10 |
| Oxadixyl + mancozeb | 3b | 4 | 23.8 | 4 |
| Propamocarb-HCl | 2a | 1 = | 16.5 | 3 |
| Propamocarb-HCl + mancozeb | NT[d] | | | |
| Metalaxyl | 8cde | 7 | 48.3 | 6 |
| Metalaxyl + mancozeb | NT | | | |
| Fosetyl-Al + mancozeb | 12e | 10 = | 79.1 | 11 |
| Chlorothalonil | 12e | 10 = | 68.6 | 9 |
| Mancozeb | 5c | 5 | 29.2 | 5 |
| Copper oxychloride | 6cd | 6 | 58.0 | 7 |
| Cupric hydroxide | 9de | 8 = | 65.8 | 8 |

[a]Final Percentage Disease Severity expressed as a function of cotyledon and primary leaf infection where FPDS = (C + 4P).2; C, percentage of cotyledon area infected; P, percentage of primary leaf area infected. FPDS is a measure of the leaf area infected as a percentage of the maximum potential infection at the termination of the trial. [b]Area under disease progress curve, calculated by trapezoid integration according to Berger (1988). [c]Mean separation in columns by Duncan's multiple range test, $p = 0.05$. [d]NT, Not tested. [e]=, Numerically equal in rank.

Table 3. Effect of fungicide applied as a foliar spray, during spring 1989, on the disease severity of downy mildew on cabbage seedlings grown in containerized trays

| Treatment | FPDS[a] | Rank | AUDPC[b] | Rank |
|---|---|---|---|---|
| Cymoxanil + mancozeb | 3a[c] | 1 | 22.7a | 1 |
| Cymoxanil + chlorothalonil | NT[d] | | | |
| Oxadixyl | 96c | 5 =[e] | 418.7de | 7 = |
| Oxadixyl + mancozeb | 13b | 2 = | 88.1b | 2 = |
| Propamocarb-HCl | 90c | 5 = | 413.5de | 7 = |
| Propamocarb-HCl + mancozeb | 60c | 5 = | 257.1cd | 5 |
| Metalaxyl | 96c | 5 = | 710.3e | 10 = |
| Metalaxyl + mancozeb | 96c | 5 = | 676.5e | 10 = |
| Fosetyl-Al + mancozeb | 75c | 5 = | 354.4d | 6 |
| Chlorothalonil | 23b | 2 = | 126.7bc | 4 |
| Mancozeb | 96c | 5 = | 424.0de | 7 = |
| Copper oxychloride | NT | | | |
| Cupric hydroxide | 19b | 2 = | 92.8b | 2 = |

[a–e]As in *Table 2*

Table 4. Effect of fungicide applied as a foliar spray, during summer 1990, on the disease severity of downy mildew on cabbage seedlings grown in containerized trays

| Treatment | FPDS[a] | Rank | AUDPC[b] | Rank |
|---|---|---|---|---|
| Cymoxanil + mancozeb | 44a[c] | 1 | 163.7a | 1 |
| Cymoxanil + chlorothalonil | NT[d] | | | |
| Oxadixyl | NT | | | |
| Oxadixyl + mancozeb | 56ab | 2 =[e] | 309.0bc | 2 = |
| Propamocarb-HCl | 53ab | 2 = | 350.8cd | 4 = |
| Propamocarb-HCl + mancozeb | NT | | | |
| Metalaxyl | NT | | | |
| Metalaxyl + mancozeb | NT | | | |
| Fosetyl-Al + mancozeb | NT | | | |
| Chlorothalonil | 66ab | 2 = | 396.3cd | 4 = |
| Mancozeb | 59ab | 2 = | 285.8bc | 2 = |
| Copper oxychloride | NT | | | |
| Cupric hydroxide | 56ab | 2 = | 350.8cd | 4 = |

[a–e]As in *Table 3*

cides, confirming the results of Samoucha and Cohen (1984, 1986, 1989) and Gisi, Binder and Rimbach (1985). Contrary to the findings of Davies and Wafford (1988), in these trials chlorothalonil, applied as a foliar spray at the onset of disease, did provide some control.

The existence of metalaxyl-resistant strains of *P. parasitica* in the Natal area was verified. Both metalaxyl and the combination of metalaxyl plus mancozeb proved to be ineffective against the mixed strains tested. At present, cross-resistance is not evident to either oxadixyl, a related

phenylamide chemical, or to the other fungicides tested.

In all cases, cymoxanil plus mancozeb provided the best control, followed closely by oxadixyl plus mancozeb. It is interesting to note that the protectant fungicides mancozeb, chlorothalonil and cupric hydroxide gave comparable results. The lack of efficacy of fosetyl-Al plus mancozeb was surprising as fosetyl-Al has excellent systemic activity against Oomycetes (Cohen and Coffey, 1986) and is registered for control of downy mildew of grapes. Further trials, however, have revealed that this may be the result of limited uptake due to the waxy nature of cabbage leaves, and may be solved by the use of an appropriate adjuvant (M. D. Laing and T. F. Brophy, unpublished).

The different levels of disease observed during the three seasons is consistent with the normal seasonal fluctuation of *P. parasitica* experienced in crucifer nurseries. Downy mildew is more prevalent in spring and autumn when large variations in temperature result in high relative humidities and free moisture, both necessary for infection. At low levels of disease, protectant fungicides provided good control. However, as the disease intensity increased, the control provided by protectants became increasingly inadequate and only systemics provided effective control.

To ensure the continued effectivity of the active systemic fungicides it is suggested that a combination of both the major strategies identified as minimizing the development of resistance to phenylamide fungicides be adopted. The first entails the use of premixed formulations, preferably with components (Gisi, 1988), of unrelated chemicals having different modes of action, either systemic or protectant (Kable and Jeffrey, 1980). The second strategy is to use similarly unrelated chemicals in alternation (Skylakakis, 1981).

The major differences between the procedures described in this paper and other fungicide work is directly related to the nature of the system adopted. The utilization of a containerized seedling system has a number of distinct experimental advantages. Discrete yet flexible units facilitate the adoption of an optimal biometrical design (randomized complete blocks). The plots (individual trays) are compact and yet contain a sufficient number of individuals to obtain a good normal distribution. This ensures that the size of the trial remains manageable and yet can include a statistically representational number of repetitions. In addition, the use of seedlings as host plants reduces the duration of the experiments, thus allowing a greater number of trials to be conducted in any one growing season.

The inoculation technique used was specifically devised for *P. parasitica* as it is an obligate biotroph, but can be used successfully for any pathogen reliant on air or splash spore dispersal. Inoculation by artificial introduction of infected seedlings, but allowing natural spread of a pathogen, is thought to provide levels of disease more similar to those occurring in a typical nursery situation. Elimination of spore collection and culture *in vitro* not only saves time but also eliminates the chance of reduced pathogenicity of the inoculum. Provided that the inoculator plants have sufficient sporulating tissue and are evenly dispersed, subsequent inoculum levels should be comparable.

A number of successive sprays were applied to elucidate general fungicidal activity over a prolonged period and to allow efficacy to be tested through a number of plant developmental stages, i.e. from cotyledon through to tertiary and quaternary leaf formation.

Similarly, sequential disease assessments were made in order to evaluate disease progress. AUDPC provides a composite figure of disease levels and hence fungicide efficacy, for comparative purposes. Final percentage disease level (FPDS) was found to be an inadequate parameter for separation of fungicide treatments that effectively delayed the onset of disease but did not reduce the final disease level (*Table 3*). This form of control assessment has particular value in the context of seedling diseases, such as downy mildew, where early infection of the cotyledon can lead to severe stunting or death of the seedling, whereas infection of the true leaves seldom causes serious damage (Channon *et al.*, 1970). For similar reasons, a visual rating scale was used to determine disease severity, rather than merely assessing disease incidence. For the reasons given, the procedures described provide a valuable tool for screening a number of chemicals concurrently.

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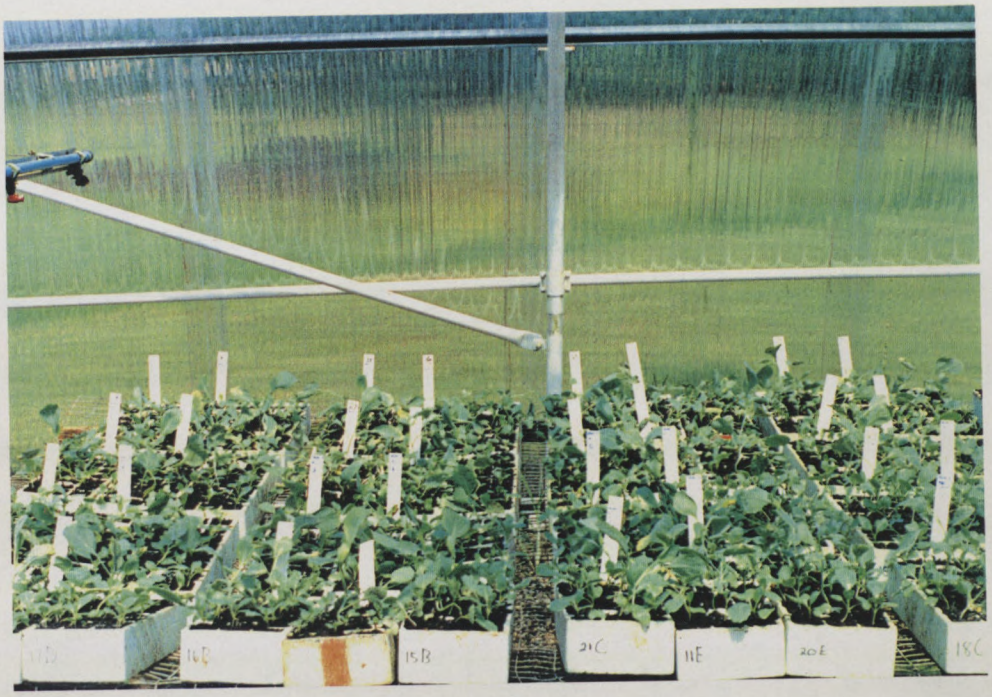
Received 29 October 1990
Revised 20 August 1991
Accepted 20 August 1991

APPENDIX 2: PHOTOGRAPHIC REPRESENTATION OF TRIAL OUTLAY




TRIAL 1

AUTOMATIC IRRIGATION SYSTEM OF TRIAL 1

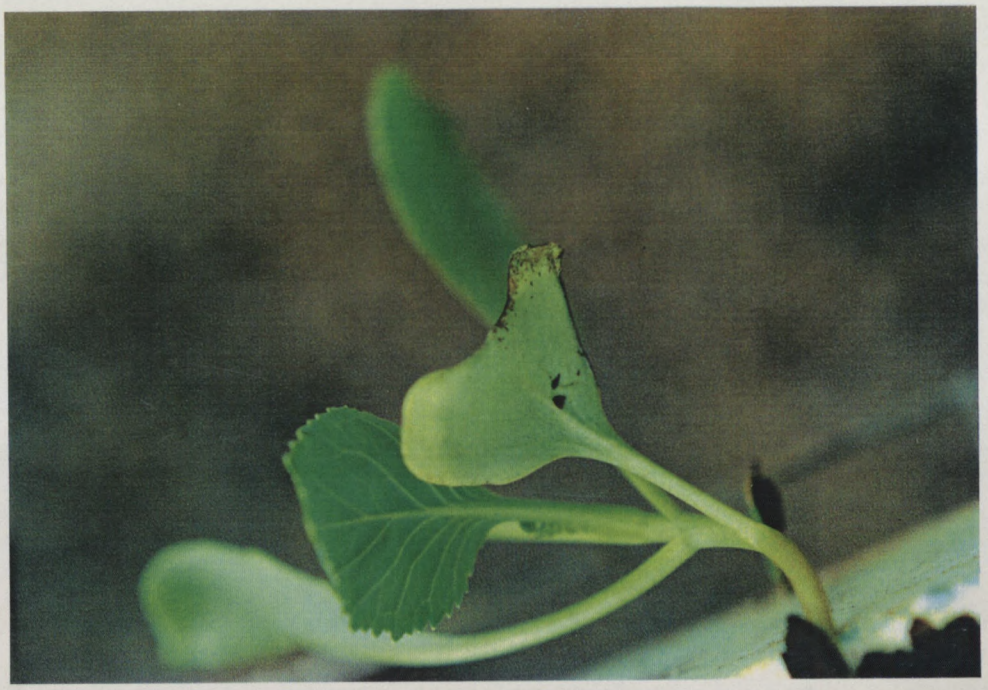
TRIAL 2 WITH MANUAL IRRIGATION

TRIAL 3

APPENDIX 3: PHOTOGRAPHIC REPRESENTATION OF DISEASE PROGRESSION

STAGE 1: COTYLEDON SHOWING NO DISEASE

STAGE TWO: ONSET OF DISEASE ( 6% LAI)



STAGE 3: INCREASED DISEASE SPREAD ( 20% LAI)

STAGE 4: SEVERE INFECTION WITH PROMINENT SPORULATION
( 55% LAI)

STAGE 5: 100% LAI

Appendix 4: Tables of results

Table 9: Trial 1P (Prophylactic)

Repetition 1

| DATE | 3CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 18/01 | 7.6 | 2.2 | 16.3 | 16.2 | 1.1 | 13.3 | 3.0 | 11.6 |
| 19/01 | 13.0 | 5.8 | 39.3 | 23.5 | 3.7 | 15.4 | 6.3 | 24.1 |
| 20/01 | 40.4 | 27.5 | 70.7 | 55.5 | 16.1 | 32.7 | 18.1 | 48.5 |
| 21/01 | 52.7 | 33.0 | 87.1 | 66.9 | 28.9 | 83.1 | 26.5 | 65.9 |
| 22/01 | 63.8 | 34.9 | 89.5 | 83.7 | 42.9 | 95.2 | 35.0 | 83.8 |
| 23/01 | 66.5 | 42.3 | 98.4 | 82.1 | 54.5 | 100 | 48.2 | 93.9 |
| 24/01 | 70.0 | 55.0 | 97.7 | 95.5 | 86.7 | 100 | 67.8 | 99.5 |
| 25/01 | 84.7 | 60.4 | 99.1 | 96.0 | 95.9 | 100 | 92.6 | 100 |
| 26/01 | 84.0 | 68.3 | 100 | 99.3 | 98.9 | 100 | 97.1 | 100 |

Repetition 2

| DATE | 3CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 18/01 | 12.5 | 5.9 | 2.5 | 7.9 | 3.5 | 8.8 | 2.6 | 20.8 |
| 19/01 | 14.0 | 11.2 | 17.0 | 14.9 | 10.8 | 15.3 | 4.3 | 25.9 |
| 20/01 | 41.9 | 25.0 | 45.8 | 52.5 | 63.1 | 52.6 | 51.3 | 48.0 |
| 21/01 | 70.0 | 42.1 | 48.5 | 58.1 | 69.4 | 61.9 | 66.7 | 59.8 |
| 22/01 | 81.4 | 60.3 | 57.6 | 64.7 | 74.2 | 79.5 | 78.0 | 75.4 |
| 23/01 | 84.1 | 66.7 | 68.7 | 84.9 | 82.0 | 97.4 | 83.1 | 89.9 |
| 24/01 | 90.8 | 76.6 | 68.0 | 95.0 | 91.9 | 99.5 | 85.9 | 94.0 |
| 25/01 | 98.2 | 83.5 | 81.4 | 98.1 | 89.6 | 100 | 87.2 | 98.9 |
| 26/01 | 99.9 | 90.8 | 83.6 | 99.5 | 92.4 | 100 | 90.0 | 100 |

Repetition 3

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 18/01 | 6.5 | 16.9 | 4.8 | 10.2 | 14.1 | 8.9 | 5.0 | 11.3 |
| 19/01 | 11.1 | 34.0 | 33.0 | 28.0 | 25.0 | 11.4 | 11.5 | 28.6 |
| 20/01 | 22.1 | 59.1 | 30.9 | 54.9 | 65.6 | 57.9 | 52.7 | 63.1 |
| 21/01 | 41.5 | 77.2 | 35.7 | 69.5 | 79.5 | 74.8 | 86.5 | 81.8 |
| 22/01 | 66.0 | 76.8 | 64.9 | 89.3 | 92.9 | 88.2 | 95.1 | 89.4 |
| 23/01 | 84.5 | 95.8 | 73.8 | 94.9 | 95.7 | 97.2 | 95.6 | 95.4 |
| 24/01 | 91.8 | 96.3 | 79.6 | 100 | 97.3 | 100 | 98.4 | 98.5 |
| 25/01 | 95.8 | 97.1 | 82.9 | 100 | 98.2 | 100 | 100 | 100 |
| 26/01 | 99.5 | 99.5 | 85.9 | 100 | 98.9 | 100 | 100 | 100 |

Table 10: Trial 1C (Cure)

Repetition 1

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 18/01 | 13.0 | 17.8 | 16.2 | 19.5 | 12.4 | 18.5 | 22.1 | 17.6 |
| 19/01 | 13.2 | 28.4 | 54.1 | 29.0 | 33.5 | 18.9 | 51.0 | 10.0 |
| 20/01 | 55.2 | 53.9 | 33.3 | 48.5 | 55.6 | 37.9 | 57.4 | 24.7 |
| 21/01 | 97.2 | 74.1 | 47.8 | 58.0 | 57.1 | 52.7 | 79.7 | 27.3 |
| 22/01 | 99.3 | 79.8 | 71.5 | 67.2 | 62.3 | 92.9 | 34.9 | 79.0 |
| 23/01 | 99.8 | 87.7 | 76.3 | 76.1 | 94.9 | 76.9 | 98.4 | 63.1 |
| 24/01 | 100 | 91.2 | 83.5 | 85.0 | 98.4 | 83.9 | 98.7 | 81.2 |
| 25/01 | 100 | 95.0 | 94.2 | 92.9 | 99.4 | 89.3 | 100 | 88.5 |
| 26/01 | 100 | 96.9 | 98.5 | 100 | 99.9 | 94.6 | 100 | 98.3 |

Repetition 2

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 18/01 | 10.8 | 65.0 | 19.9 | 22.0 | 11.4 | 10.5 | 28.1 | 25.9 |
| 19/01 | 19.7 | 68.0 | 14.6 | 32.5 | 15.1 | 55.0 | 40.3 | 38.0 |
| 20/01 | 82.8 | 82.9 | 34.1 | 45.7 | 70.1 | 66.2 | 77.5 | 48.5 |
| 21/01 | 73.3 | 91.5 | 57.9 | 63.9 | 89.2 | 94.0 | 100 | 73.2 |
| 22/01 | 84.1 | 97.3 | 73.3 | 77.7 | 92.1 | 95.3 | 100 | 90.1 |
| 23/01 | 82.2 | 95.4 | 76.5 | 90.1 | 93.8 | 100 | 100 | 95.7 |
| 24/01 | 82.3 | 99.1 | 88.9 | 83.4 | 97.3 | 100 | 100 | 96.9 |
| 25/01 | 95.5 | 99.7 | 94.4 | 98.6 | 99.0 | 100 | 100 | 99.2 |
| 26/01 | 98.0 | 100 | 93.6 | 100 | 100 | 100 | 100 | 100 |

Repetition 3

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 18/01 | 12.0 | 31.5 | 58.6 | 46.6 | 32.3 | 15.5 | 13.5 | 10.1 |
| 19/01 | 72.0 | 48.2 | 43.0 | 38.2 | 26.5 | 19.4 | 38.0 | 37.6 |
| 20/01 | 42.1 | 66.8 | 81.1 | 66.2 | 40.8 | 43.4 | 44.3 | 38.8 |
| 21/01 | 53.0 | 89.4 | 90.5 | 76.2 | 67.4 | 50.2 | 58.0 | 66.7 |
| 22/01 | 70.9 | 93.2 | 96.1 | 85.2 | 69.5 | 71.5 | 68.5 | 77.4 |
| 23/01 | 73.0 | 97.7 | 98.6 | 88.5 | 76.5 | 87.6 | 82.3 | 85.9 |
| 24/01 | 78.1 | 99.8 | 98.6 | 93.3 | 83.9 | 93.2 | 88.1 | 93.6 |
| 25/01 | 78.0 | 100 | 100 | 96.6 | 88.5 | 96.3 | 92.4 | 96.4 |
| 26/01 | 92.4 | 100 | 100 | 99.5 | 97.7 | 96.9 | 96.1 | 96.4 |

Table 11: Trial 1PC (Prophylactic & Cure)

Repetition 1

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 18/01 | 7.8 | 11.0 | 12.2 | 17.8 | 3.2 | 6.9 | 6.1 | 13.5 |
| 19/01 | 22.9 | 10.9 | 21.6 | 20.0 | 16.2 | 15.7 | 15.0 | 13.0 |
| 20/01 | 18.1 | 2o.7 | 52.0 | 48.6 | 81.1 | 41.9 | 68.1 | 65.0 |
| 21/01 | 39.3 | 33.3 | 45.8 | 68.9 | 95.7 | 41.1 | 88.6 | 69.9 |
| 22/01 | 42.0 | 47.3 | 70.1 | 80.9 | 98.4 | 82.9 | 18.9 | 93.5 |
| 23/01 | 66.5 | 49.8 | 70.8 | 90.6 | 95.0 | 91.7 | 79.4 | 85.1 |
| 24/01 | 72.1 | 58.8 | 85.1 | 98.7 | 98.4 | 100 | 88.0 | 97.0 |
| 25/01 | 85.0 | 79.8 | 90.0 | 99.9 | 99.5 | 100 | 91.9 | 97.0 |
| 26/01 | 89.2 | 89.1 | 90.7 | 100 | 100 | 100 | 96.1 | 99.4 |

Repetition 2

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 18/01 | 9.7 | 14.3 | 13.7 | 17.6 | 17.8 | 15.6 | 1.6 | 9.1 |
| 19/01 | 15.5 | 33.8 | 16.1 | 25.3 | 9.3 | 16.8 | 14.3 | 23.2 |
| 20/01 | 22.8 | 68.4 | 24.2 | 60.8 | 79.7 | 55.9 | 70.5 | 76.2 |
| 21/01 | 52.8 | 72.7 | 33.0 | 77.3 | 90.7 | 83.0 | 85.3 | 74.5 |
| 22/01 | 64.5 | 93.5 | 49.9 | 76.6 | 97.0 | 94.2 | 94.8 | 91.6 |
| 23/01 | 75.4 | 90.5 | 62.2 | 85.0 | 97.1 | 94.1 | 92.5 | 95.7 |
| 24/01 | 80.2 | 95.7 | 79.5 | 94.7 | 98.9 | 99.8 | 94.0 | 96.0 |
| 25/01 | 91.6 | 96.2 | 96.7 | 99.8 | 99.9 | 100 | 99.8 | 96.6 |
| 26/01 | 95.1 | 100 | 100 | 100 | 100 | 100 | 100 | 97.0 |

Repetition 3

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 18/01 | 7.0 | 12.2 | 19.2 | 5.1 | 5.4 | 11.5 | 5.6 | 15.4 |
| 19/01 | 17.7 | 40.7 | 22.1 | 6.7 | 7.0 | 21.1 | 18.5 | 21.6 |
| 20/01 | 51.7 | 63.5 | 62.4 | 29.1 | 74.3 | 42.5 | 88.6 | 28.7 |
| 21/01 | 72.1 | 99.1 | 92.0 | 52.5 | 75.3 | 51.8 | 93.3 | 58.2 |
| 22/01 | 81.6 | 99.2 | 95.4 | 61.5 | 91.7 | 96.8 | 92.0 | 85.5 |
| 23/01 | 82.5 | 100 | 96.4 | 57.4 | 91.1 | 89.4 | 96.0 | 96.5 |
| 24/01 | 89.5 | 100 | 100 | 71.5 | 95.0 | 92.6 | 99.1 | 99.1 |
| 25/01 | 96.3 | 100 | 100 | 79.9 | 100 | 95.6 | 100 | 99.9 |
| 26/01 | 99.7 | 100 | 100 | 84.2 | 100 | 98.0 | 100 | 100 |

Table 12: Trial 2P (Prophylactic)

Repetition 1

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 28/03 | 16.9 | 20.2 | 20.2 | 10.4 | 10.5 | 5.5 | 2.7 | 4.3 |
| 29/03 | 29.2 | 25.0 | 34.4 | 28.9 | 14.1 | 13.3 | 5.3 | 11.1 |
| 30/03 | 47.0 | 48.0 | 53.2 | 40.8 | 26.3 | 28.4 | 9.6 | 21.9 |
| 01/04 | 62.0 | 56.6 | 75.6 | 58.9 | 24.4 | 25.0 | 4.9 | 45.0 |
| 02/04 | 62.8 | 70.2 | 85.5 | 68.0 | 45.9 | 59.4 | 9.4 | 51.5 |
| 04/04 | 99.7 | 99.7 | 99.5 | 97.5 | 98.6 | 90.0 | 17.9 | 93.3 |
| 06/04 | 100 | 100 | 100 | 100 | 100 | 97.0 | 17.9 | 96.7 |

Repetition 2

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 28/03 | 26.4 | 20.4 | 14.5 | 8.0 | 4.9 | 11.0 | 3.3 | 3.2 |
| 29/03 | 29.0 | 25.8 | 31.3 | 23.0 | 23.0 | 20.9 | 5.2 | 8.0 |
| 30/03 | 51.0 | 46.5 | 45.3 | 37.7 | 25.2 | 33.0 | 6.1 | 12.0 |
| 01/04 | 58.3 | 61.3 | 67.3 | 61.9 | 37.5 | 51.5 | 12.0 | 45.8 |
| 02/04 | 73.3 | 68.5 | 66.2 | 73.6 | 57.9 | 64.8 | 15.8 | 39.6 |
| 04/04 | 99.4 | 99.7 | 98.9 | 99.0 | 93.7 | 89.5 | 23.9 | 84.5 |
| 06/04 | 100 | 100 | 100 | 100 | 100 | 100 | 23.5 | 93.0 |

Repetition 3

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 28/03 | 21.0 | 16.8 | 10.2 | 14.3 | 5.3 | 9.5 | 3.8 | 1.6 |
| 29/03 | 34.8 | 36.5 | 26.6 | 31.8 | 19.0 | 29.9 | 10.4 | 5.6 |
| 30/03 | 50.9 | 47.0 | 44.9 | 43.0 | 33.2 | 44.5 | 16.9 | 8.2 |
| 01/04 | 63.3 | 66.3 | 66.3 | 51.8 | 38.0 | 57.8 | 29.0 | 28.2 |
| 02/04 | 61.7 | 74.6 | 64.8 | 73.3 | 72.3 | 67.2 | 30.2 | 27.8 |
| 04/04 | 99.7 | 99.7 | 99.0 | 99.0 | 98.2 | 92.0 | 43.9 | 70.4 |
| 06/04 | 100 | 100 | 100 | 100 | 100 | 100 | 39.8 | 78.4 |

Table 13: Trial 2C (Cure)

Repetition 1

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 28/03 | 13.8 | 11.5 | 7.0 | 9.7 | 7.1 | 3.1 | 14.9 | 14.9 |
| 29/03 | 40.5 | 30.5 | 25.4 | 25.5 | 23.6 | 17.5 | 23.6 | 22.0 |
| 30/03 | 54.3 | 47.3 | 31.6 | 41.0 | 38.7 | 38.5 | 34.4 | 39.5 |
| 01/04 | 81.8 | 74.8 | 45.3 | 54.6 | 43.0 | 56.7 | 58.6 | 54.6 |
| 02/04 | 85.4 | 81.0 | 72.8 | 79.1 | 68.9 | 75.7 | 76.3 | 77.6 |
| 04/04 | 99.8 | 99.5 | 98.8 | 99.8 | 97.8 | 98.9 | 98.6 | 95.0 |
| 06/04 | 100 | 100 | 100 | 100 | 100 | 100 | 99.3 | 97.3 |

Repetition 2

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 28/03 | 9.8 | 7.8 | 6.7 | 9.8 | 5.8 | 4.7 | 14.5 | 12.6 |
| 29/03 | 34.0 | 29.5 | 17.1 | 24.4 | 22.7 | 17.0 | 22.7 | 18.7 |
| 30/03 | 47.5 | 74.6 | 35.3 | 30.8 | 37.2 | 34.2 | 39.8 | 27.0 |
| 01/04 | 71.8 | 77.5 | 58.8 | 60.5 | 43.0 | 53.0 | 48.9 | 57.7 |
| 02/04 | 78.8 | 81.5 | 81.7 | 67.2 | 63.6 | 73.1 | 69.0 | 66.7 |
| 04/04 | 99.4 | 99.0 | 99.0 | 99.4 | 92.7 | 99.2 | 98.8 | 98.0 |
| 06/04 | 100 | 100 | 100 | 100 | 100 | 100 | 99.6 | 99.3 |

Repetition 3

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 28/03 | 23.3 | 6.8 | 5.0 | 9.5 | 5.2 | 16.8 | 18.2 | 8.3 |
| 29/03 | 29.0 | 26.5 | 23.0 | 17.7 | 20.6 | 26.2 | 27.2 | 12.4 |
| 30/03 | 45.3 | 46.2 | 39.1 | 34.0 | 34.6 | 40.1 | 50.6 | 27.0 |
| 01/04 | 73.8 | 67.0 | 55.3 | 60.5 | 56.5 | 56.8 | 61.9 | 41.3 |
| 02/04 | 76.8 | 69.6 | 73.5 | 68.5 | 55.3 | 68.5 | 75.7 | 55.4 |
| 04/04 | 98.8 | 99.1 | 99.3 | 99.4 | 93.4 | 95.8 | 99.3 | 96.3 |
| 06/04 | 100 | 100 | 100 | 100 | 98.9 | 100 | 97.0 | 99.3 |

Table 14: Trial 2PC (Prevention & Cure)

Repetition 1

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 28/03 | 16.5 | 16.8 | 8.3 | 8.5 | 3.4 | 2.4 | 3.1 | 11.9 |
| 29/03 | 18.4 | 29.4 | 16.4 | 21.2 | 9.7 | 16.3 | 10.3 | 17.5 |
| 30/03 | 27.3 | 42.1 | 30.2 | 37.7 | 20.9 | 31.7 | 16.7 | 20.0 |
| 01/04 | 55.4 | 49.2 | 39.8 | 40.5 | 25.3 | 45.8 | 20.0 | 51.4 |
| 02/04 | 56.9 | 70.0 | 61.2 | 61.9 | 34.9 | 61.0 | 32.0 | 51.8 |
| 04/04 | 99.3 | 98.9 | 97.6 | 98.8 | 71.3 | 96.2 | 25.9 | 96.1 |
| 06/04 | 100 | 100 | 100 | 100 | 90.2 | 100 | 32.3 | 100 |

Repetition 2

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 28/03 | 11.1 | 11.8 | 6.7 | 6.3 | 4.0 | 6.7 | 2.0 | 3.0 |
| 29/03 | 28.1 | 22.1 | 27.4 | 17.5 | 16.6 | 27.3 | 4.6 | 8.6 |
| 30/03 | 35.3 | 40.7 | 33.1 | 38.3 | 32.1 | 46.6 | 8.7 | 16.9 |
| 01/04 | 59.2 | 44.1 | 39.8 | 49.6 | 43.3 | 63.6 | 10.7 | 35.7 |
| 02/04 | 65.0 | 67.2 | 66.0 | 73.0 | 61.6 | 72.2 | 17.8 | 49.5 |
| 04/04 | 98.7 | 99.0 | 99.7 | 97.5 | 84.5 | 97.8 | 16.3 | 94.6 |
| 06/04 | 100 | 100 | 100 | 100 | 100 | 100 | 16.6 | 99.3 |

Repetition 3

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 28/03 | 15.7 | 8.0 | 5.6 | 3.6 | 2.7 | 10.2 | 4.2 | 3.5 |
| 29/03 | 27.5 | 14.4 | 16.0 | 15.0 | 14.9 | 18.9 | 7.8 | 9.5 |
| 30/03 | 39.4 | 34.3 | 38.3 | 31.2 | 31.2 | 39.2 | 17.1 | 36.1 |
| 01/04 | 43.6 | 44.8 | 40.3 | 43.5 | 38.3 | 58.3 | 18.3 | 43.0 |
| 02/04 | 62.3 | 62.1 | 61.2 | 64.0 | 56.3 | 67.5 | 26.3 | 47.5 |
| 04/04 | 99.2 | 97.3 | 98.8 | 98.4 | 80.7 | 97.8 | 26.7 | 84.3 |
| 06/04 | 100 | 100 | 100 | 100 | 94.2 | 100 | 32.9 | 93.4 |

Table 15: Trial 3P (Prophylactic)

Repetition 1

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | |
|---|---|---|---|---|---|---|---|---|
| 02/05 | 18.2 | 22.1 | 24.2 | 7.4 | 12.5 | 10.2 | 5.5 | |
| 03/05 | 22.5 | 24.2 | 39.2 | 28.4 | 16.1 | 11.8 | 8.2 | |
| 04/05 | 55.1 | 55.9 | 48.2 | 43.8 | 26.6 | 24.4 | 11.2 | |
| 05/05 | 62.9 | 57.2 | 72.4 | 52.2 | 29.2 | 27.1 | 13.4 | |
| 06/05 | 74.1 | 69.2 | 83.2 | 62.2 | 52.0 | 49.4 | 15.3 | |
| 08/05 | 99.7 | 99.9 | 97.6 | 98.4 | 97.7 | 91.1 | 16.8 | |
| 10/05 | 100 | 100 | 99.8 | 100 | 100 | 97.0 | 18.4 | |

Repetition 2

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | |
|---|---|---|---|---|---|---|---|---|
| 02/05 | 28.2 | 26.3 | 20.5 | 7.4 | 7.1 | 9.3 | 2.9 | |
| 03/05 | 36.4 | 28.1 | 39.2 | 27.1 | 21.8 | 25.6 | 4.9 | |
| 04/05 | 64.2 | 33.5 | 46.2 | 41.1 | 23.3 | 34.2 | 6.2 | |
| 05/05 | 64.2 | 66.8 | 65.1 | 67.7 | 35.6 | 50.9 | 11.1 | |
| 06/05 | 78.9 | 73.5 | 72.2 | 72.4 | 60.2 | 67.2 | 13.4 | |
| 08/05 | 99.1 | 97.8 | 97.8 | 100 | 93.2 | 91.3 | 19.8 | |
| 10/05 | 100 | 100 | 100 | 100 | 98.8 | 100 | 22.0 | |

Repetition 3

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 02/05 | 18.3 | 17.2 | 12.5 | 15.8 | 4.1 | 7.7 | 1.8 | 5.3 |
| 03/05 | 41.2 | 28.9 | 24.4 | 18.9 | 5.6 | 7.8 | 3.6 | 9.8 |
| 04/05 | 50.1 | 33.5 | 41.2 | 46.2 | 29.8 | 16.9 | 14.6 | 21.3 |
| 05/05 | 56.3 | 59.8 | 61.5 | 56.3 | 34.2 | 33.5 | 25.8 | 40.1 |
| 06/05 | 58.8 | 72.0 | 68.2 | 68.2 | 70.2 | 58.5 | 28.7 | 49.2 |
| 08/05 | 89.2 | 94.1 | 88.7 | 89.0 | 92.3 | 89.3 | 42.3 | 72.5 |
| 10/05 | 99 | 100 | 98.4 | 99.2 | 100 | 100 | 42.2 | 91.2 |

Table 16: Trial 3C (Cure)

Repetition 1

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 02/05 | 12.3 | 10.1 | 4.9 | 3.8 | 4.8 | 1.5 | 10.9 | 10.5 |
| 03/05 | 35.6 | 25.8 | 21.5 | 22.6 | 20.2 | 15.4 | 20.5 | 19.4 |
| 04/05 | 48.2 | 42.6 | 27.8 | 37.5 | 32.6 | 36.4 | 31.2 | 37.4 |
| 05/05 | 78.2 | 70.8 | 42.5 | 51.6 | 40.8 | 53.6 | 55.5 | 51.4 |
| 06/05 | 81.8 | 78.6 | 70.1 | 76.7 | 66.2 | 73.4 | 72.5 | 75.1 |
| 08/05 | 94.8 | 94.2 | 93.7 | 94.4 | 92.5 | 94.3 | 93.6 | 92.3 |
| 10/05 | 100 | 100 | 99.3 | 100 | 98.7 | 100 | 97.2 | 97.5 |

Repetition 2

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 02/05 | 7.5 | 5.6 | 4.8 | 7.4 | 2.9 | 2.9 | 12.3 | 10.4 |
| 03/05 | 31.8 | 27.2 | 15.3 | 22.7 | 20.2 | 15.1 | 20.7 | 15.6 |
| 04/05 | 44.2 | 70.5 | 33.5 | 30.2 | 38.1 | 30.5 | 37.4 | 25.2 |
| 05/05 | 69.4 | 75.2 | 56.3 | 58.2 | 40.7 | 50.9 | 45.4 | 54.8 |
| 06/05 | 75.2 | 79.6 | 80.4 | 64.5 | 60.8 | 71.1 | 64.8 | 62.1 |
| 08/05 | 94.8 | 95.2 | 95.1 | 94.7 | 89.7 | 94.6 | 93.2 | 93.1 |
| 10/05 | 99.5 | 100 | 100 | 98.4 | 95.6 | 100 | 98.7 | 100 |

Repetition 3

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 02/05 | 20.2 | 4.7 | 3.6 | 7.2 | 3.5 | 13.5 | 15.8 | 5.6 |
| 03/05 | 27.4 | 24.0 | 21.4 | 15.4 | 18.3 | 24.1 | 25.4 | 10.7 |
| 04/05 | 42.7 | 44.9 | 37.5 | 31.5 | 31.6 | 39.1 | 47.8 | 24.8 |
| 05/05 | 72.6 | 65.3 | 53.4 | 57.1 | 54.2 | 54.7 | 59.2 | 39.6 |
| 06/05 | 74.5 | 67.7 | 70.0 | 64.8 | 55.2 | 67.8 | 74.8 | 51.5 |
| 08/05 | 96.5 | 97.2 | 97.4 | 97.8 | 92.6 | 93.4 | 97.5 | 95.9 |
| 10/05 | 100 | 100 | 100 | 100 | 96.9 | 98.7 | 100 | 100 |

Table 17: Trial 3PC (Prevention & Cure)

Repetition 1

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 02/05 | 14.3 | 14.3 | 5.6 | 5.1 | 1.8 | 1.2 | 3.0 | 10.4 |
| 03/05 | 16.6 | 27.4 | 14.3 | 20.8 | 7.8 | 14.6 | 9.2 | 16.3 |
| 04/05 | 25.7 | 40.5 | 29.6 | 35.6 | 18.7 | 28.5 | 14.7 | 19.5 |
| 05/05 | 53.5 | 47.8 | 37.4 | 39.9 | 24.6 | 44.1 | 21.3 | 47.4 |
| 06/05 | 53.8 | 64.2 | 60.7 | 49.8 | 30.3 | 58.7 | 26.6 | 50.6 |
| 08/05 | 97.4 | 96.6 | 95.2 | 95.6 | 71.3 | 94.2 | 29.7 | 94.2 |
| 10/05 | 100 | 100 | 98.7 | 98.5 | 92.3 | 98.7 | 30.5 | 98.8 |

Repetition 2

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 02/05 | 9.8 | 9.2 | 5.7 | 5.3 | 2.7 | 4.5 | 1.6 | 2.3 |
| 03/05 | 26.1 | 20.3 | 25.4 | 16.5 | 14.3 | 25.8 | 3.8 | 5.7 |
| 04/05 | 33.5 | 39.6 | 31.2 | 37.3 | 30.8 | 44.4 | 7.6 | 13.3 |
| 05/05 | 52.8 | 40.6 | 36.6 | 48.4 | 40.3 | 60.4 | 11.2 | 36.2 |
| 06/05 | 62.5 | 68.3 | 64.4 | 69.3 | 59.5 | 70.2 | 13.8 | 48.9 |
| 08/05 | 97.3 | 98.4 | 96.6 | 95.4 | 85.2 | 95.4 | 14.5 | 95.3 |
| 10/05 | 100 | 100 | 100 | 100 | 94.6 | 98.7 | 14.4 | 99.9 |

Repetition 3

| DATE | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| 02/05 | 10.5 | 5.6 | 4.8 | 2.6 | 3.0 | 10.0 | 3.6 | 2.8 |
| 03/05 | 26.8 | 12.2 | 14.8 | 13.6 | 13.6 | 18.2 | 4.8 | 8.9 |
| 04/05 | 35.8 | 30.5 | 34.9 | 28.7 | 29.6 | 35.6 | 14.4 | 30.8 |
| 05/05 | 42.6 | 42.3 | 40.5 | 41.2 | 36.6 | 54.4 | 20.3 | 40.7 |
| 06/05 | 59.2 | 60.7 | 58.9 | 62.5 | 54.6 | 65.6 | 24.8 | 45.6 |
| 08/05 | 97.1 | 95.6 | 97.7 | 97.9 | 86.3 | 95.8 | 24.8 | 88.7 |
| 10/05 | 100 | 98.3 | 100 | 100 | 90.4 | 98.7 | 29.7 | 97.9 |

Table 18: TRIAL 1P

| | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| Rep 1 | 436.9 | 294.2 | 640.0 | 561.0 | 378.7 | 583.1 | 344.5 | 572.0 |
| Rep 2 | 536.6 | 413.8 | 430.1 | 522.0 | 529.0 | 560.6 | 517.1 | 460.4 |
| Rep 3 | 465.8 | 594.5 | 446.2 | 591.7 | 610.7 | 583.9 | 593.9 | 612.5 |

Trial 1C

| | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| Rep 1 | 615.2 | 567.5 | 518.1 | 515.5 | 574.1 | 478.5 | 639.2 | 387.8 |
| Rep 2 | 574.3 | 716.4 | 496.5 | 552.9 | 612.3 | 665.8 | 681.9 | 604.6 |
| Rep 3 | 519.3 | 660.9 | 687.9 | 617.3 | 518.1 | 517.8 | 526.4 | 549.7 |

Trial 1PC

| | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| Rep 1 | 394.4 | 350.7 | 485.9 | 566.5 | 635.9 | 526.8 | 501.0 | 577.0 |
| Rep 2 | 455.2 | 607.9 | 418.5 | 578.3 | 631.5 | 601.6 | 605.5 | 606.9 |
| Rep 3 | 544.8 | 658.6 | 627.9 | 403.3 | 587.1 | 533.3 | 645.1 | 553.7 |

Table 19: Trial 2P

| | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| Rep 1 | 540.7 | 544.4 | 600.6 | 530.8 | 436.1 | 435.6 | 89.0 | 440.7 |
| Rep 2 | 560.3 | 546.0 | 548.2 | 535.0 | 462.4 | 487.1 | 119.9 | 388.8 |
| Rep 3 | 551.5 | 569.5 | 538.1 | 541.7 | 497.7 | 521.8 | 231.1 | 303.7 |

Trial 2C

| | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| Rep 1 | 611.2 | 577.9 | 512.6 | 544.2 | 507.8 | 525.6 | 535.0 | 527.3 |
| Rep 2 | 575.2 | 605.8 | 535.0 | 520.2 | 486.6 | 514.6 | 519.4 | 505.1 |
| Rep 3 | 512.6 | 545.7 | 528.8 | 518.5 | 483.0 | 525.9 | 557.9 | 459.9 |

Trial 2 PC

| | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| Rep 1 | 493.3 | 531.9 | 477.6 | 494.1 | 341.9 | 478.9 | 180.7 | 464.8 |
| Rep 2 | 523.1 | 511.6 | 502.1 | 513.1 | 455.4 | 544.8 | 100.9 | 425.5 |
| Rep 3 | 510.2 | 485.3 | 486.8 | 484.3 | 425.8 | 518.4 | 171.1 | 423.6 |

Table 20: Trial 3P

| | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| Rep 1 | 560.2 | 552.0 | 591.7 | 518.2 | 451.6 | 421.7 | 110.5 | 460.1 |
| Rep 2 | 595.5 | 547.4 | 564.7 | 548.2 | 459.8 | 508.7 | 105.4 | 373.7 |
| Rep 3 | 522.4 | 527.0 | 511.5 | 508.8 | 461.6 | 428.4 | 214.8 | 383.9 |

## Trial 3C

| | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| Rep 1 | 579.4 | 550.6 | 486.1 | 517.5 | 479.0 | 504.9 | 505.8 | 508.2 |
| Rep 2 | 551.1 | 585.5 | 518.3 | 499.3 | 466.7 | 493.8 | 491.9 | 480.2 |
| Rep 3 | 557.6 | 532.5 | 513.9 | 500.4 | 470.8 | 511.9 | 547.5 | 458.8 |

## Trial 3PC

| | 5CH | 9CH | 15CH | 30CH | OPT | PHY | S151 | CON |
|---|---|---|---|---|---|---|---|---|
| Rep 1 | 478.5 | 512.4 | 464.3 | 462.9 | 332.4 | 463.0 | 176.5 | 451.5 |
| Rep 2 | 505.7 | 504.6 | 486.2 | 499.6 | 441.0 | 527.7 | 87.5 | 420.2 |
| Rep 3 | 493.5 | 468.4 | 476.8 | 474.4 | 546.2 | 501.9 | 157.8 | 425.5 |