# Spatial and temporal patterns of witches' broom disease on proteas

Rosemary Newton

Witches' broom on *Protea nitida* at Jonkershoek

Botany Honours Thesis
University of Cape Town
Supervisor: Prof. W.J. Bond
1996

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# Abstract

Witches' broom is a visible fasciation occurring in many proteas, believed to be caused by a mycoplasma. The vector is thought to be the mite *Aceria proteae*, which is commonly found in witches' brooms. Witches' broom is a problem for *Protea* farmers in the cut flower industry. Once the disease appears in a field, it spreads rapidly, is difficult to control, and is rarely eradicated. Assumptions underlying disease control guidelines are that the disease is localized to a portion of the plant, and that the infection spreads rapidly from a point source. This study investigated witches' broom infection at different spatial and temporal scales, to test these assumptions, and gain a fuller understanding of the characteristics, transmission and spread of the disease. Two infected natural populations and an infected agricultural population were used for pattern analysis, to determine spatial scales of disease spread. Experimental field studies involved phytosanitary procedure testing, and laboratory studies involved seed germination from infected and non-infected plants; mite observation and data compilation on the distribution of the infection within species. Disease behaviour following broom removal in an agricultural population, and the spread of disease in a natural population following broom introduction was monitored. The study revealed two types of disease spread. The first is a very slow rate of spread at a localized scale within the plant and between neighbouring plants, which does not agree with the first assumption of control guidelines. The second is long distance dispersal of the disease between isolated populations. Long distance dispersal of mite vector is suggested to be in the form of a cloud, which, on settling, will produce multiple infections on many plants. This strategy contrasts with the type of disease dispersal assumed for witches' broom, in which the infection of a single plant is followed by secondary disease spread outward from the initial point of infection. The assumption that the disease is localized and is not systemic is partially correct. However this localization is from the point of disease infection, rather than symptom expression, which does not necessarily correspond. Unfortunately it is not currently possible to determine the point of disease infection. In view of these findings, it is suggested that farmers remove the whole stem of a plant with witches' broom and completely remove plants that are heavily infected with the disease.

## Introduction

Disease has always been prevalent in natural plant communities, and most likely plays an important role as a selective agent in plant evolution by mediating plant-plant interactions and structuring plant communities. Plant diseases are caused by three major groups of pathogens, namely fungi, bacteria and viruses (Burdon, 1987). Mycoplasmas and mycoplasma-like organisms (MLO's) also cause plant disease, and according to Manners (1982), appear to be related to bacteria. Yet because they are obligate pathogens like viruses, and cause diseases resembling viral diseases, mycoplasmas are conventionally grouped with viruses (Manners, 1982). Disease control efforts to date have focused primarily on agricultural communities, because of the economic importance of healthy plants in human society, and the devastation that disease can cause in agricultural stands (Burdon, 1987).

The study of plant disease can be approached from two different but complementary angles; namely that of process or pattern. Some important disease processes include pathogen transmission, entry and infection (Manners, 1982). Disease patterns may be studied at a variety of spatial or temporal scales (Burdon *et al.*, 1995). Disease processes and patterns vary substantially between different pathogens and plant hosts. Both approaches to the study of disease are equally valid and important, and as they are closely linked (Turner, 1989), the study of one can lead to an understanding of and predictions about the other.

The process of disease transmission from host to host may be direct, or may indirectly involve a vector (Begon *et al.*, 1990), many of which are arthropods (Manners, 1982 & Borror *et al.*, 1989). Coevolution of the disease or vector with the host inevitably takes place over time, and this may lead to host pathogen specificity. Yet over evolutionary time, disease resistance in certain host strains may occur (Burdon, 1987). Both host-pathogen specialization and disease resistance in host strains control disease patterning.

Disease patterns, such as temporal and spatial spread within a population, can also be indicative of disease processes. Despite the paucity of disease studies in natural communities, these compared with agricultural studies would be beneficial, by leading to a fuller understanding of the disease, and the most effective means of disease control.

In this study I investigated patterns of infection of witches' broom, a common disease on proteas, in both natural and cultivated populations of proteas.

## Case Study: Witches' broom on proteas

### The disease

Witches' broom is a common term describing the visible effects of fasciation in plants caused by a variety of pathogens. Witches' broom fasciation is observed to occur on many members of the genus *Protea* in South Africa (Knox-Davies *et al.*, 1988 and Forsberg, 1993). Disease symptoms on *Protea* species are a characteristic thick bushy growth, consisting of poorly developed shoots with thin stems and minute, often distorted leaves (Myburgh & Rust, 1971). The broom is produced by excessive proliferation from normally dormant floral or vegetative axial buds (Dorrington, 1988 and van Broembsen, 1989). Single branches on plants or whole plants can be affected (Myburgh & Rust, 1971). Diseased plants can only be identified by symptom observation, and the physical change of the plant is irreversible (Dorrington, 1988).

### The pathogen

The cause, or agent, of the disease has not yet been identified (Forsberg, 1993), but is thought to be a microscopic mycoplasma or MLO. These organisms are between 50 - 800 nm in diameter and lack cell walls (Smith, 1974 and Manners, 1982). They are confined to the phloem and are transmitted from plant to plant by phloem-feeding vectors, such as insects and mites (Smith, 1977; Manners, 1982 and Dorrington, 1988). Mycoplasmas remain in the plant throughout the plant's life.

A toxin or possibly hormone from the causal agent is believed to stimulate the *Protea* bud to divide and subdivide to form the witches' broom growth (Myburgh & Rust, 1971). Infection of the plant may occur as early as in the young seedling stage (Meynhardt, 1986). The time lag between infection and symptom expression is unknown. Polymerase chain reaction studies of the causal agent of witches' broom are currently in progress by Ms E. Venter at the Directorate of Plant and Quality Control in Stellenbosch. Hereafter, for simplicity, the causal agent, although not confirmed, will be referred to as a mycoplasma.

### The vector

The mode of transmission of the mycoplasma to other parts of the plant and to other plants is unknown. Some authors (Myburgh & Rust, 1975; Coetzee *et al.*, 1986 and Dorrington, 1988) have suggested, due to close mite-plant association, that transmission may be with the eriophyid mite, *Aceria proteae*. This suggestion is supported by the fact that other members of the Eriophyidae are known vectors for plant pathogens (Dorrington, 1988).

Eriophyid mites are usually very small (< 0.2 mm in length) and worm-like. They are soft-bodied and have a thin cuticle, and are therefore prone to water loss. Typically they develop through the life cycle stages of egg, first-nymph and second-nymph before reaching the adult stage. The colonizing success of these mites (fitting r-strategist characteristics) can be attributed to features such as parthenogenetic reproduction, widespread dispersal capabilities and a high intrinsic rate of increase (Rodriguez & Rodriguez, 1987).

Although eriophyid mites cause relatively slight feeding injury to the young plant tissue compared to other mites, excessive feeding may destroy the food resource, causing the leaf microenvironment that formerly had a high transpiration rate to become increasingly dry. On leaf dehydration, mite behaviour changes from a sedentary to a more active phase. If new food sources are not encountered by migration or dispersal, the mites will perish from dehydration or starvation (Rodriguez & Rodriguez, 1987). Rates of dispersal, and time intervals between one food source and the next, are also unknown.

*Aceria proteae* mites specifically are colourless, banana-shaped and microscopically small (Rust & Myburgh, 1976). They have been found under bracts in dormant leaf buds, in flower buds (unopened inflorescences) and in witches' brooms (Coetzee *et al.*, 1986). It is believed that the witches' broom resulting from the introduction of the mycoplasma into the plant by the mite becomes the breeding ground for successive generations of mites (Meynhardt, 1986).

Mite reproduction takes place throughout the whole year, but especially quickly in the summer (Rust & Myburgh, 1976). The larvae and adult mites feed on new broom growth, before dispersing to other parts of the plant and other plants (Meynhardt, 1986). Dispersal mechanisms for eriophyid mites are undocumented, but related tetranychid mites disperse by launching into the wind (Hoy *et al.*, 1984 in Rodriguez & Rodriguez, 1987), which may be as low as 8 km/h for successful dispersal (Boykin & Campbell, 1984 in Rodriguez & Rodriguez, 1987). Dispersal of *A. proteae* has been proposed to be by wind, humans, and possibly on birds (Myburgh & Rust, 1971).

### Witches' broom on cultivated proteas

Witches' broom is a problem for *Protea* farmers in the cut flower industry, as infected plants often do not produce economically acceptable flower crops (Coetzee *et al.*, 1989) due to reduced flower production (Myburgh *et al.*, 1973). Flowers that are produced are often spoiled by the closeness of an axillary witches' broom infection.

Published guidelines (Rust, 1984 and Coetzee *et al.*, 1989) for disease eradication and control in flower farms are:

1) Prevention, by ensuring diseased plants are not sold from nurseries;
2) Mite control, through regular miticide application;
3) Destruction of heavily infested adult plants and diseased seedlings;
4) Pruning of light infestations on mature plants; and,
5) Burial or destruction of infested material by burning.

Yet despite these guidelines for disease eradication being adhered to by farmers, once the disease has appeared in a field, it spreads rapidly, is difficult to control, and is rarely eradicated. The disease is also patchy both within and between plants and species. Furthermore, within mixed plantations, one species may become severely infected while the other species remains uninfected. Thus there is still a great deal about the disease that remains a mystery. It is far from clear how the disease spreads, both spatially and temporally, and how it affects its hosts.

## Questions addressed in the study

The aims of this study were to elucidate more about witches' broom, in terms of its characteristics, transmission, patterns of infection and spread in both natural populations and an agricultural population. Patterns of infection were studied at three different spatial scales: within a plant, between neighbouring plants, and at a regional scale. The temporal scale within these studies ranges between a few months and a few years to evolutionary time.

Disease control procedures are based on assumptions that the disease is localized to the portion of the plant with visible symptoms, and that disease spreads quickly within a population from one or several point sources. In this study I used both natural patterns of disease distribution and experiments to test these assumptions. I asked questions at the following scales of observation:

### Within a plant

1) Is the disease (mycoplasma) localized to the area of infection or systemic throughout the plant?
   a) Is disease transmitted from the vegetative broom through the phloem to the flower head and reproductive seed, and thus to the next generation?
   b) Is the failure of witches' broom removal in agricultural populations due to the mycoplasma being systemic within the plant?
3) Is within-plant infection related to plant size, previous, or current infection?
4) Is the disease transmitted mechanically by secateurs?

### Between plants in a population

1) Is infection influenced by the infection, density or size of the neighbours?
2) What spatial patterns of disease spread are observed following:
   a) Witches' broom introduction to an uninfected population?
   b) Witches' broom removal from an infected population following published guidelines?

### At a regional scale

1) Which Proteaceae species are affected?
2) Is there an evolutionary pattern of disease in the family and genus?

# Methods and Results

## Study sites

### Table Mountain

The Valley of the Red Gods on Table Mountain (33°58' S, 18°23' E) contained a natural *Protea cynaroides* (L.) L. population both in flower and in various stages of cone production. Approximately half of the population displayed witches' broom. It was therefore an ideal site to collect seed for germination, to test whether the disease could be transmitted from the parent plant to the progeny through seed. Fruit (hereafter loosely referred to as seed) collection took place in an area of approximately 1 km$^2$.

### Constantiaberg

A population of *Protea lepidocarpodendron* (L.) L. plants, south of Bokkemanskloof, on the road to Constantiaberg (34°02' S, 18°23' E), were studied for within-plant interactions and neighbour effects. This population occupied an area of about 80 m X 250 m. The plants had all regenerated from seed following the summer burning of a fire break in 1991. Over half of the plants had witches' broom, and the site was thus ideal to examine disease distribution within plants and near neighbours.

### Jonkershoek

On 17 February 1996, a large portion of the Jonkershoek valley, consisting of natural vegetation dominated by the resprouting *Protea nitida* Mill., was burnt in an uncontrolled fire. Two subsites within this burned area (33°59' S, 18°57' E) were chosen to study witches' broom.

#### *Jonkershoek site 1*

This site contained a mature population of *P. nitida*, which had been partially burned by the fire. This population showed many burnt "skeletons" of witches' broom infection, and surviving infections where the fire was not as severe. Within-plant interactions and neighbour effects were studied. This site was also used to investigate the possibility of mechanical transmission of the disease by secateurs.

*Jonkershoek site 2*

A neighbouring population of *P. nitida* had been severely burned by the fire, and there was no live witches' broom remaining in the population. The plants were observed for the return of the disease with the resprouting of new shoots, but on the continued absence of the disease from the population for over 100 days, witches' brooms were introduced from plants at Jonkershoek site 1, to observe patterns of disease spread.

### Devon Valley

The farm Etshwaleni (33°54' S, 18°48' E), located in Devon Valley just outside Stellenbosch, was chosen to examine the methods and relative effectiveness of witches' broom removal in an agricultural population, as well as the spatial pattern of disease return over time. *P. cynaroides* is extensively cultivated from seed on a 17500 $m^2$ section of the farm. Plants are arranged in 8 blocks of 6 X 174 plants. Plants within a block are separated from one another by 1 m on the long axis and 1.5 m on the short axis. Blocks are separated from one another by 2.5 m. At the time of the study, the plants in the field were two years old or younger, as plants that died were replaced with younger seedlings. Plants had been treated with an insecticide for mites a few months prior to the experiment.

## Within-plant studies

### Is the disease localized or systemic?

*Transmission from vegetative (witches' broom) to reproductive (seed) tissue?*

An experiment was conducted to test whether the mycoplasma may be transmitted via phloem from a witches' broom to a flower head, and to seeds of the next generation. Flower heads containing mature *P. cynaroides* seeds were collected from the Table Mountain site on 30 April 1996. Twelve flower heads, one per plant, were collected from each of:

1) Infected plants - flower heads on a stem directly above a broom;
2) Infected plants - flower heads on a stem without visible infection; and,
3) Plants with no visible symptoms of witches' broom.

Flower heads were oven-dried in brown paper bags for 36 hours at 40°C. Seeds were then removed from the flower heads, and sorted according to weight. Only 26 of the 36 flower heads collected had viable seed. Generally speaking, seeds < 0.03 g were woody and not viable, whereas seeds > 0.04 g almost always contained an embryo. Viable seeds were set out to germinate in Petri dishes in a solution of 0.5% benlate (fungicide). Seeds took approximately 2 weeks to germinate. Once the seedlings had a radical of between 5 and 15 mm, approximately 100 per collection sample, with a maximum of 15 per flower head, were planted in seedling trays. The seedling trays were kept in a mite-free greenhouse. Development of witches' broom in a mite-free environment would indicate transmission of the disease in the seed, and not by mites. The seedlings were watered three times a week, and observed for the development of witches' broom.

*RESULTS* : No seedlings showed symptoms of witches' broom at the time of the report write-up, after 75 to 100 days. Personal observation of approximately 6 month old seedlings of *P. nitida*, germinated from seed at Jonkershoek site 1 subsequent to the fire, showed signs of witches' broom. According to Mr. P. van Rensburg, the manager of the farm in Devon Valley, *P. cynaroides* seedlings of a few months old have been observed with witches' broom. The fact that infection can occur in young seedlings, but has not done so in the experiment, suggest that witches' broom is not transmitted between generations via phloem, although it is still too early to conclude this for certain. Observation of the seedlings will therefore be continued for one year to ensure adequate time is allowed for witches' broom development.

*Agricultural removal techniques successful?*

The *P. cynaroides* plantation in Devon Valley was studied to determine whether the failure to remove infection from agricultural populations using specified removal techniques was due to the removal method, or because the disease was systemic in the plant. If the disease is localized within the portion of the plant showing disease symptoms, agricultural removal of the diseased section should be effective in eradicating the disease. Ineffectual removal would result in broom regrowth. New infections on a previously infected plant might suggest the disease to be systemic.

On 15 August 1996, witches' broom was removed in a single block of infected plants by two farm workers who usually remove the growth at Etshwaleni. Prior witches' broom removal had taken place. The method of witches' broom removal was dependent on the position of the infection. Witches' broom that was terminal on a stem was removed by cutting the stem below the witches' broom with secateurs. Secateurs were sterilized after each cut in formalin. Witches' broom that was growing on a lower part of the stem or from the rootstock was removed by pulling off the growth, in order to prevent severe damage to the stem or rootstock. The resultant wounds were immediately painted with black tree sealer.

Plants in the block were mapped, and plants showing evidence of prior witches' broom infection (identified by the presence of the black tree sealer), as well as those in which witches' broom had been removed on 15 August, were noted. These plants were subsequently checked three times at 15 day intervals for regrowth of witches' broom from the removal point.

*RESULTS*: The number of plants that had been previously infected or were infected with witches' broom at the time of removal, was 7% of the 1044 plants examined. Within 45 days, 31% of the 98 localities on stems where the parasite had been removed showed witches' broom regrowth. The current methods of broom removal are therefore ineffectual. Most regrowth was from removals in which the broom had been pulled off at the base of the plant from the rootstock. This could indicate that the infected tissue, assuming the disease is localized, was not completely removed. Yet symptoms developed directly below the cut in some plants in which broom was removed from aril stems well below the broom. This might indicate that the disease was present in the whole stem. The inability to distinguish the point of infection is thus a confounding factor in determining whether the disease is localized or not. Furthermore, the lack of knowledge of the time period between infection and disease expression, makes it impossible to conclude whether the disease is localized or systemic.

The most direct approach to the question of "localized" versus "systemic" is to determine whether the mycoplasma observed in the witches' broom phloem, is present in stem phloem, both above and below the point from which the broom was growing. Eight *P. lepidocarpodendron* plants from the Constantiaberg site, half of which were visibly infected, were taken to Mrs E. Venter for Polymerase chain reaction analysis. Unfortunately at the time of write-up, this analysis was not yet complete.

### Is within-plant infection related to plant size, previous, or current infection?

Populations of *P. lepidocarpodendron* at Constantiaberg and *P. nitida* at Jonkershoek site 1 were sampled to determine whether the degree of infection of witches' broom (broom load) within a plant was related to plant size, or influenced by the absence or degree of previous infection. If the disease were systemic, it would be expected that infection before the fire would be related to the appearance of infection after the fire. Data used for this analysis was also used for the elucidation of possible interactions between neighbouring plants (refer to next section). Sampling methods were thus designed for the neighbour study, and so comprised the collection of measurements from randomly chosen target plants and their closest five neighbours.

Measurements included plant height (m), two perpendicular widths (m), and the number of witches' brooms. In the Jonkershoek population, witches' brooms were subdivided into dead skeletons (indicator of previous infection), new live infections, and burnt infections which were killed but regrowing (both indicators of current infection). The regrowing infections were not included into the "previous infection" category, because this would have biased the correlation matrix to the previous infection being more highly correlated with the current infection. The objective of this comparison was to determine whether the previous infection of witches' broom, that seemed to be eradicated by fire, would be related to the current live infection. In the Constantiaberg population the total number of witches' brooms, whether live, dying or dead (died naturally and not from fire) were counted as indicators of current broom infection, as they were all postfire infections.

Plant volume ($m^3$) was calculated from height and width measurements with the following equation:

$$\text{Volume} = \Pi * \text{height} * \text{width1} * \text{width2}$$

*RESULTS*: Table 1 shows means and standard deviations of plant size and degree of infection in *P. lepidocarpodendron* at Constantiaberg and *P. nitida* at Jonkershoek. This data was used in correlation matrices to determine the strength of relationships between witches' broom infection and plant size.

**Table 1** Means and standard deviations for plant size and broom infection on *P. nitida* at Jonkershoek (N=108) and *P. lepidocarpodendron* at Constantiaberg (N=238).

| | Jonkershoek | Constantiaberg |
|---|---|---|
| Height (m) | 2.732±0.971 | 0.846±0.212 |
| Volume ($m^3$) | 89.60±106.4 | 0.536±0.687 |
| Number of previous infections | 3.731±8.958 | |
| Number of current infections | 0.454±1.307 | 0.303±0.730 |

A correlation matrix (STATISTICA, Version 5.1) was constructed for both Jonkershoek and Constantiaberg populations to determine whether there was any relationship between plant size and previous infection to current infection, and if so, which of these variables explained the highest proportion of the variance in current infection levels within a plant. Plant height and log volume were used as indicators of plant size, the log of the number of dead witches' broom as indicators of previous infection (Jonkershoek data only), and the log of the number of live witches' broom (Jonkershoek) or total witches' broom (Constantiaberg) as indicators of current infection.

The correlation matrix constructed for *P. lepidocarpodendron* at Constantiaberg revealed no significant relationship between plant size and infection (Table 2).

**Table 2** Correlation table with $r^2$ values for witches' broom infection and host plant size of *P. lepidocarpodendron* at Constantiaberg (N=238). Neither correlation was statistically significant ($p > 0.1$).

| | Plant height | Log plant volume |
|---|---|---|
| Log number of current infections | -0.113 | -0.012 |

The results of the correlation matrix are hardly surprising, as there was little variation in plant size (refer to Table 1): they were all the same age, having grown from seed following the burning of the fire break five years previously. If broom infection was related to plant size, this would only be noticeable in an older population in which greater variation in plant size would exist.

Thus, correlation matrices were constructed for the older *P. nitida* at Jonkershoek (Table 3). Two matrices were constructed. The first (N = 108) showed that the past infection of witches' broom was significantly correlated to plant size, but that current infection was not related to past infection. This data included two outliers with uncharacteristically high previous infections (53 and 73 brooms per plant respectively), in comparison with the mean previous infection 3.075 ± 4.381 (N=106). A second analysis with the exclusion of these points was done, to ensure that results of the first correlation analysis were not being influenced by these points. Probability values for the second matrix, however, remained unchanged.

**Table 3** Correlation table with $r^2$ values for broom infection and host plant size of *P. nitida* at Jonkershoek (N = 108). The second correlation excluded the two heavily infected *P. nitida* plants (N = 106). Values with an asterisk were significant at $p < 0.0001$.

| | Plant height | Log plant volume | Log current infections |
|---|---|---|---|
| Log previous infections (N = 108) | 0.420 ** | 0.448 ** | 0.151 |
| Log previous infections (N = 106) | 0.380 ** | 0.414 ** | 0.019 |

In the Jonkershoek population, previous infection of witches' broom was strongly related to plant size. Plant volume (log transformed) was better related to infection than plant height. Post-burn (current) infection was however not correlated with pre-burn (previous) broom loads, suggesting that new infections, rather than systemic reappearance of infection, was taking place.

The pattern of parasite loads within plants can also reveal information on disease spread. A Poisson distribution of witches' broom would indicate that infection by one broom is independent of other infections. A clumped distribution would suggest that infection by a broom increases the risk of parasite spread within a host plant. A Poisson distribution was therefore fitted to the parasite load (number of infections) of the plant, and tested for goodness of fit with a Chi-squared test. A good fit of the Poisson to the data would indicate random distribution of infection, whereas a poor fit would indicate a non-random distribution of the disease within the plant. The Chi-squared test (df = 2) was highly significant for both the previous and current Jonkershoek and total Constantiaberg witches' broom infections (Table 4), indicating a poor fit of the Poisson distribution to the data.

**Table 4** Chi-squared values obtained when testing for a goodness of fit of the Poisson distribution to the broom loads within the plant (outliers excluded) at Jonkershoek (N=106) and Constantiaberg (N=238). All values were significant at $p < 0.0001$.

| | Chi-squared value |
|---|---|
| Jonkershoek previous infection | 66.71 |
| Jonkershoek current infection | 91.87 |
| Constantiaberg total infection | 32.67 |

In all instances broom infections were clumped in relation to random expectation (refer to Figure 1 for an example of the previous infection of witches' broom at Jonkershoek).

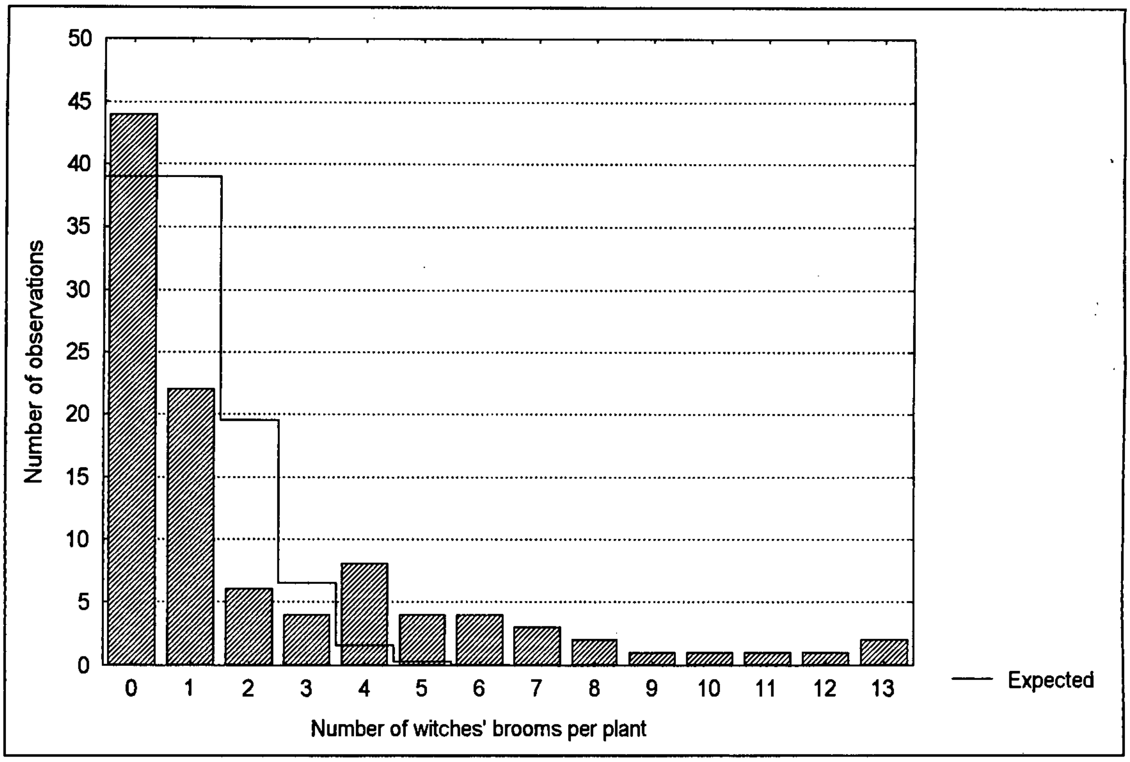


**Figure 1** Graph of the number of dead brooms within a plant at Jonkershoek (hatched bars) and the expected Poisson distribution had the infection been random.

It is clear that the presence of infection within a plant in both the Constantiaberg and Jonkershoek populations does influence the broom load, and that a non-random patterning of witches' broom occurs within plants. Interestingly, *P. nitida* (resprouter) has higher Chi-squared values than *P. lepidocarpodendron* (reseeder), indicating the disease to be more clumped within a plant in the resprouter than the reseeder. The higher clumping in the resprouter may suggest that the disease is systemic in the plant.

## Transmission via secateurs?

The population of *P. nitida* plants at Jonkershoek site 1 was used in an experiment to test the hypothesis that the disease could be transmitted from a witches' broom to an uninfected shoot in the same plant by mycoplasma transferal in the sap, or mite transferal on secateurs. Experiments were designed to mimic witches' broom removal in an agricultural situation.

On 9 June 1996, forty witches' brooms from twenty randomly chosen infected plants (two witches' brooms per plant) within the study site were removed with secateurs. After each broom removal, a cut was made through the broom, and then a shoot on the same plant was cut just above an axillary bud. The bud just below the cut was marked with wool for easy identification. The cut shoots were then regularly checked for axillary bud development.

*RESULTS*: No growth from the axillary buds took place during the 4½ months in which the plants were observed. Until growth from these buds occurs, no conclusions can be drawn as to whether witches' broom may be transmitted from one plant to the next by secateurs.

### Between plants in a population

How is infection influenced by neighbour plant characteristics?

Populations of *P. nitida* and *P. lepidocarpodendron* at Jonkershoek site 1 and Constantiaberg, respectively, were sampled to determine the influence of neighbouring plant characteristics (size, density and degree of infection) on the degree of infection of the target plant. Target plants were chosen randomly from the population, and the height and two perpendicular widths recorded. The number of live witches' brooms (live + regrowing) and dead witches' brooms at Jonkershoek; and total number of witches' brooms at Constantiaberg were also noted. The same measurements were taken for the closest five neighbouring plants of the same species. Distance from each neighbour to the target plant was measured. Plant volume was calculated as in the previous section.

Variables used in a correlation matrix to determine the relationship between the target infection and neighbour plant characteristics were the log of the number of witches' brooms on the target (indicator of broom load on target plant); the mean neighbours' height and log volume (indicators of neighbour size), the log of the sum of witches' brooms on the five neighbours (indicator of neighbour infection) and the mean distance of neighbours from the target plant (indicator of neighbour density).

Variables that best predicted target infection were explored using correlation and regression analysis. A forward stepwise multiple regression was used (STATISTICA, Version 5.1) to determine the relative importance of neighbour characteristics in explaining the pattern of infection in the target plant.

## *RESULTS*:

### *Constantiaberg*

The mean and standard deviation of neighbour distance to the target plant was 1.035 m ± 0.794 m. Plant size and infection statistics for this site are presented in Table 1. Neighbour interactions were studied in 39 target plants and five neighbours per target at this site. Correlation analysis revealed no significant relationship between neighbouring plant size or density with the infection on the target plant. However, neighbour infection was correlated with infection on target plants (Table 5).

**Table 5** $R^2$ values of a correlation table for neighbour effects on parasite load of *P. lepidocarpodendron* at Constantiaberg (N=39). Values with an asterisk were significant at $p < 0.05$.

| | **Target:** | Log number of WB |
|---|---|---|
| **Neighbours:** | | |
| Log total number of WB | | 0.376* |
| Log mean plant volume | | 0.015 |
| Mean plant height | | 0.013 |
| Mean distance to target | | -0.027 |

A forward stepwise multiple regression (F = 2 to enter) was used to determine the order of importance of neighbour characteristics in explaining the pattern of infection in the target plant. The multiple regression indicated a significant relationship (p=0.018, N=39) of the log of the target number of witches' brooms with the log of the total neighbour witches' brooms. No other variables were included in the final model. Thus at Constantiaberg, neighbour infection had the strongest influence on the target infection.

*Jonkershoek site 1*

The mean and standard deviation of neighbour distance to the target plant was 5.042 m ± 3.592 m. Plant size and infection statistics for this site are presented in Table 1. Neighbour characteristics between 18 target plants and five neighbours per target at the Jonkershoek site were studied. Correlation analysis revealed no significant relationship of any of the neighbour characteristics with the broom load on the target plant. The two outliers (refer to previous section) were again strongly affecting relationships between target and neighbouring plants, so the analysis was repeated with performed the outlier data excluded (Table 6).

**Table 6** Correlation table of $r^2$ values between broom infection levels on a target plant and neighbour characteristics in *P. nitida* at Jonkershoek (N=18), and with the groups containing the two outliers excluded (N=16). Values with an asterisk were significant ($p < 0.1$).

| **Target:** | Log no. of WB<br>N = 18 | Log no. of WB<br>N = 16 |
|---|---|---|
| **Neighbours:** | | |
| Log total number of WB | 0.331 | 0.493* |
| Log mean plant volume | 0.339 | 0.144 |
| Mean plant height | 0.396 | 0.215 |
| Mean distance to target | -0.271 | -0.404 |

With the outliers excluded from the analysis, the infection on the neighbouring plants was most strongly related to the infection of the target. This corresponds with the Constantiaberg findings, in which neighbour infection was significantly related to the infection of the target plant.

A forward stepwise multiple regression (F = 2 to enter) for data excluding the outliers indicated a significant relationship (p=0.0297, N=16) of the log of the target number of witches' brooms with the log of the total neighbour witches' brooms. The mean distance of the neighbours to the target was included in the model, but was not significant. Neighbouring plant size was not included in the final model.

In both Constantiaberg and Jonkershoek populations, the parasite load on target plants was significantly correlated with the infection of the five nearest neighbours. This could indicate that dispersal and transmission takes place over short distances in the field, and that neighbouring plant infection will increase the likelihood of infection in uninfected plants.

## Spatial patterns of witches' broom?

### *Patterns of infection following witches' broom introduction*

A study site of approximately 3840 $m^2$ at Jonkershoek site 2 was selected in order to observe the patterns of disease spread in *P. nitida* on introduction of brooms to an apparently uninfected area. Plants recovered rapidly from the fire, with resprouting shoots visible a couple of weeks after the fire. Some 100 days after the fire, plants were checked within a larger area, at least 20 m perpendicular to the study site boundaries, to ensure that the study site and immediate surrounds were free of live witches' broom. Although the previous infection in the population was 0.6 brooms per tree, no new infection was observed in the area. On 9 June 1996, witches' broom was introduced from Jonkershoek site 1 to Jonkershoek site 2. Brooms were attached with string, two per plant, one on a mature shoot with leathery leaves, and the other on a young shoot with soft leaves. This was done, on the assumption that the mites might crawl out of the broom onto the nearest shoot. Witches' brooms were tied to twenty plants of different sizes and at varying heights, and the shoots marked with coloured string for identification. The area was monitored monthly for appearance of witches' broom.

*RESULTS*: The first appearance of witches' broom at the Jonkershoek site was observed 45 days after the introduction of brooms into the area. Based on the assumption that the infection was introduced by the tied brooms, this highlights the importance of the destruction of removed witches' brooms from agricultural plantations, to prevent disease spread. The time between the broom introduction on trees and the appearance of witches' broom, suggests that there is a latent period of approximately 1 month between infection and symptom expression, as plants were actively growing during this period.

A map (Figure 2) was constructed to show the spatial relationship between the plants on which brooms were tied, and the plants on which infection appeared in the 100 days since the introduction. Ten infections developed in total, each on a different tree. Three of these plants with infections had plants on which brooms were tied as nearest neighbours, although it cannot be assumed that infection was from that neighbour. Only one of the plants on which brooms were tied developed an infection. This was however on a different branch of the plant to which the broom was tied. The percentage of the population infected over the observation period was 6.81%; which can be expressed as an infection rate of 2.04% of the population per month.

Most of the new infections seemed to be generally north west of plants with tied brooms. The Jonkershoek valley (Figure 3), which runs from north west to south east, is surrounded on three sides by high mountains, thus the wind direction throughout the year would tend to be up the valley (i.e. a north west wind). Although the wind direction on the few days after brooms were tied is not known, it might well have been (at least during part of that time) a north-wester. This would support the idea that the disease, if mite-dispersed, would be by wind, as mites had only a few days to disperse before the cut broom dried out.

The behaviour of these mites on a few opened witches' broom was observed under a light microscope; however their initially observed behaviour of walking and standing did not change, and within an hour or so, they shriveled and died. The sudden exposure of the mites to the harsh light and temperature conditions under the microscope unfortunately did not mimic the slow drying of a witches' broom, and this probably led to a change in their normal behaviour.

### *Patterns of infection following witches' broom removal*

Patterns of witches' broom regrowth and appearance were monitored in the agricultural population at Devon Valley, following the removal of infection on 15 August 1996 (refer to previous section). The number of new and recurring witches' brooms on plants were subsequently noted three times at 15 day intervals to map disease spread in the field.

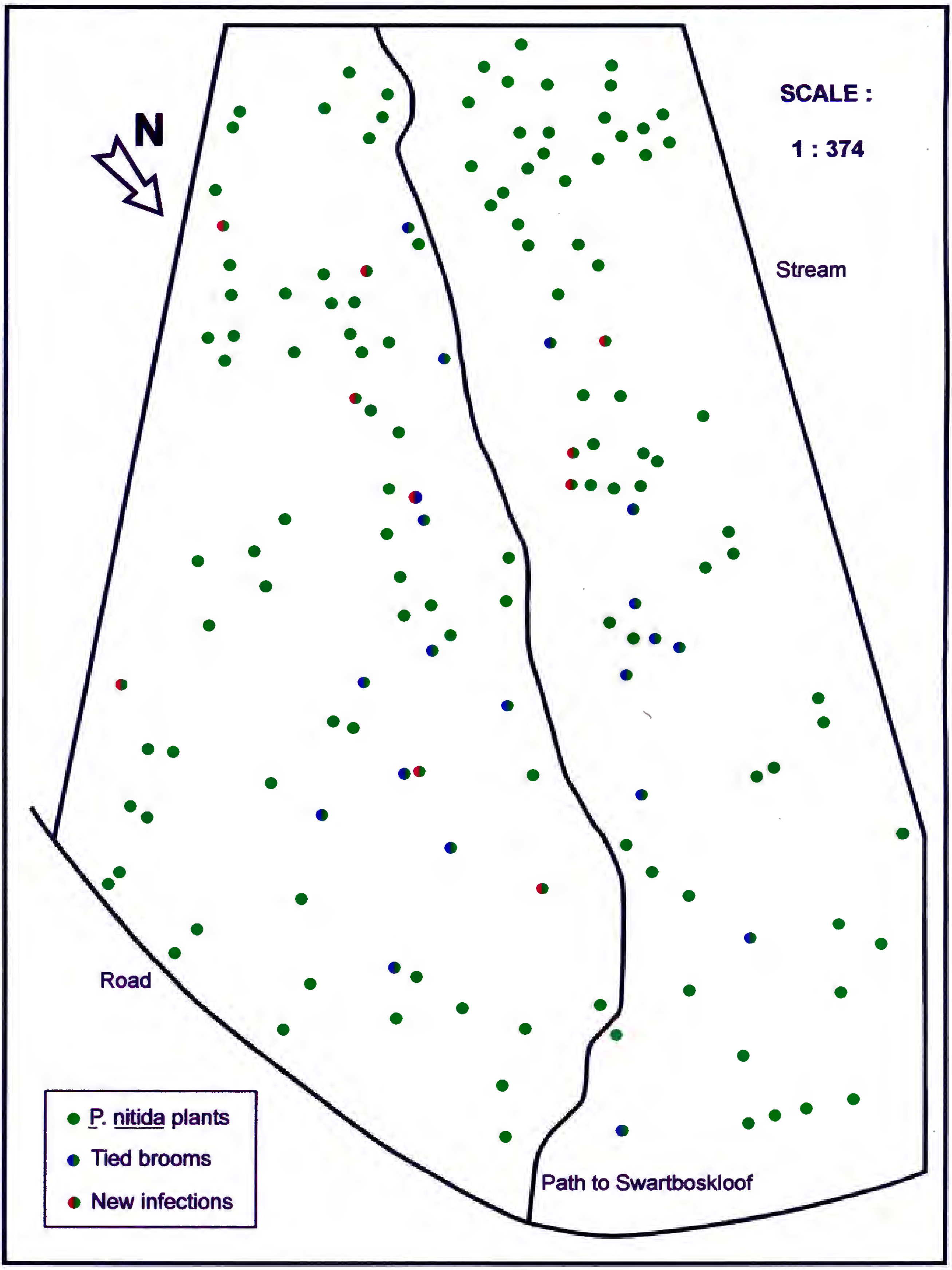


**Figure 2** Map of Jonkershoek site 2, in which P. nitida plants are shown in green. Plants on which brooms were tied are marked in blue, and plants in which new infections of witches' broom developed are marked in red.

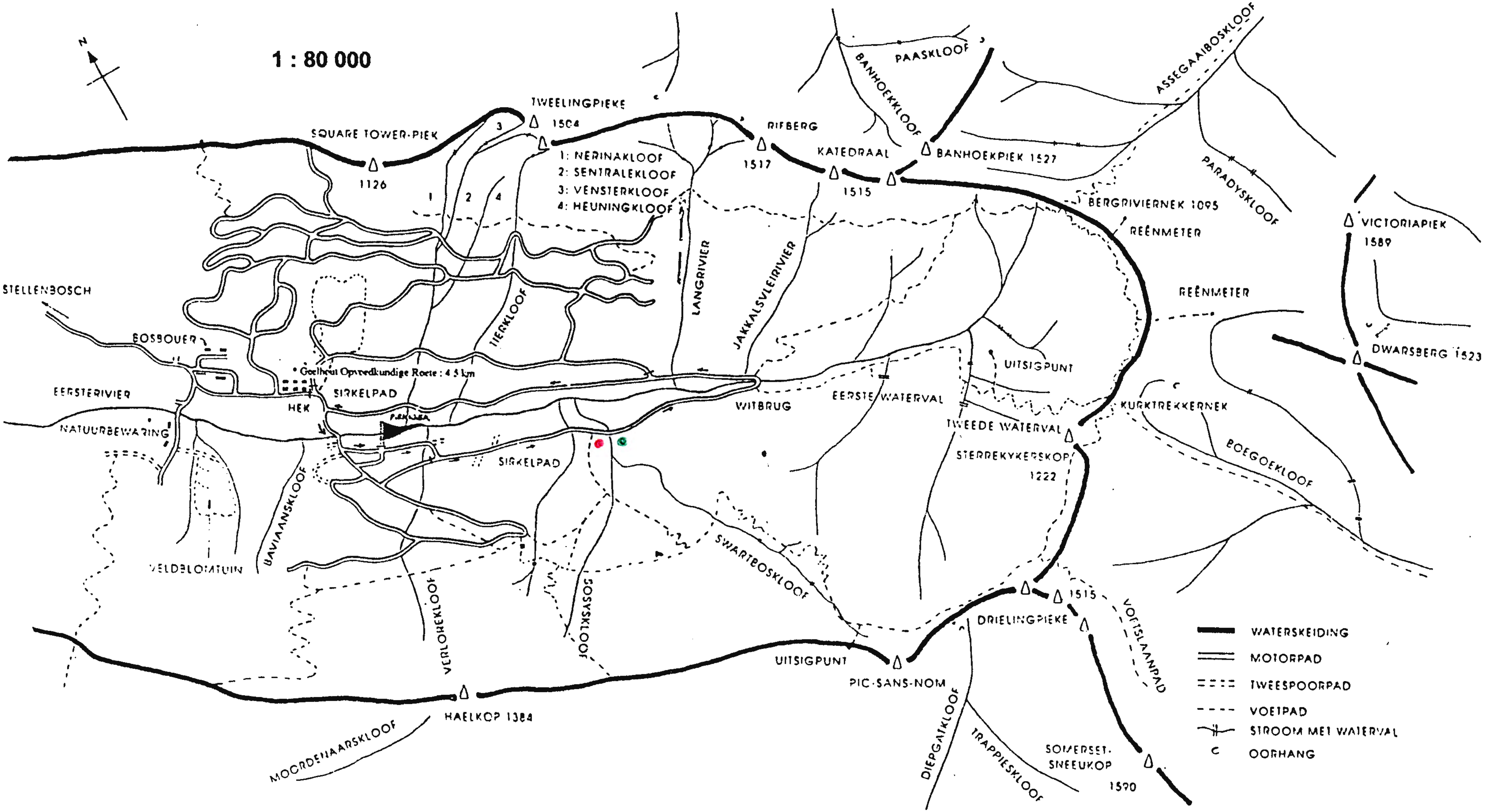
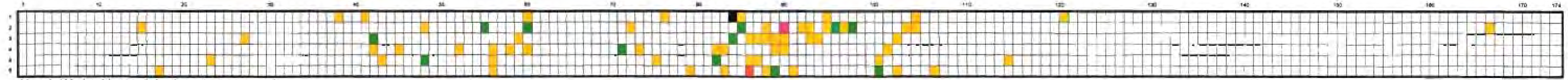
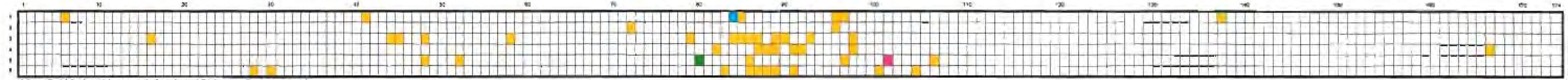


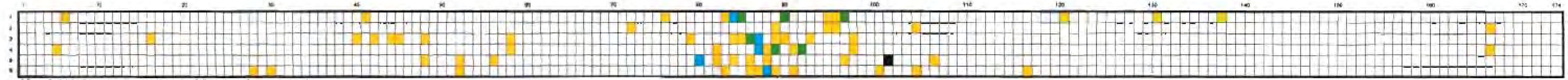
**Figure 3** Map of Jonkershoek showing the NW - SE orientation of the valley. Jonkershoek site 1 (red) and Jonkershoek site 2 (green) are marked.



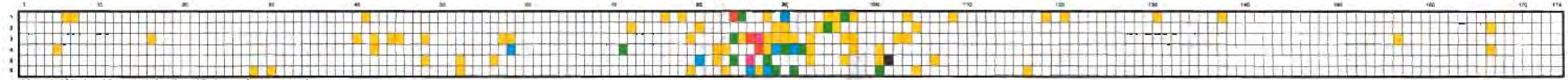
Map 1: Witches' broom infection prior to removal.



Map 2: Witches' broom infection 15 days after removal.



Map 3: Witches' broom infection 30 days after removal.



Map 4: Witches' broom infection 45 days after removal

Key: witches' broom density (number of infections per plant)

🟨 1 🟩 2 🟦 3 🟥 4 🟪 5 ■ 6 ⬛ >6

**Figure 4** Maps of witches' broom infection in a block of *Protea cynaroides* at Etshwaleni. Map A indicates witches' broom infection prior to removal; maps B, C and D respectively indicate the regrowth and spread of witches' broom infection over time.

*RESULTS*: The patterns of regrowth and new infection of witches' broom following witches' broom removal are illustrated in Figure 4. It is clear from the figure that there was a lot of infection in the centre of the field prior to broom removal. Disease returned quickly to the field in this highly diseased section, as a result of both broom regrowth from shoots where it had been previously removed, as well as the appearance of new infections - both on plants already infected as well as apparently uninfected plants.

The number of plants with witches' broom prior to removal was then graphed against the number of plants with infection at day 45 for every 10 rows (60 plants) of the field (Figure 5).

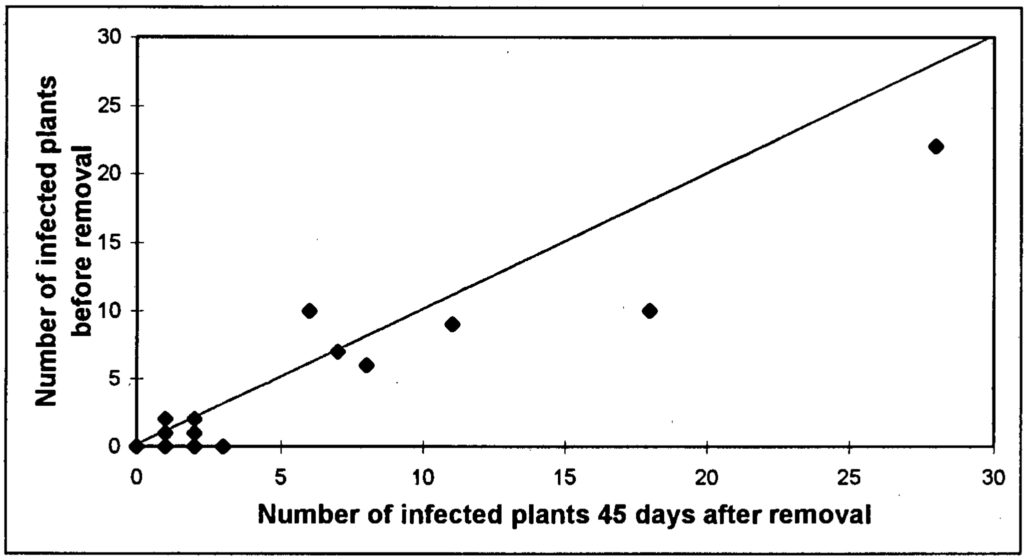


**Figure 5** Graph of the number of *P. cynaroides* plants with infection prior to removal with the number of plants with infection at day 45, for every 10 rows in the field after the start of the experiment at Etshwaleni.

Figure 5 shows that the spread of the disease to other plants in the field was slow over the 45 days of observation. The number of diseased plants 45 days after broom removal were rarely more than the number of infected plants prior to broom removal. Yet the return of the disease to the field was rapid, with 66.2% of the total number of infected plants prior to removal showing infection 15 days later.

Percentages of disease spread in terms of newly infected plants and plants increasing broom load are shown in Table 7 for dense infection (rows 81 - 105) and sparse infection (whole field excluding rows 81 -105) of the disease.

**Table 7** Monthly rate of new infection of *P. cynaroides* plants and broom load increase within infected plants expressed as a percentage for the area of dense disease infection (rows 81-105) and sparse disease infection (whole field excluding rows 81 -105).

| | Sparse infection | Dense infection |
|---|---|---|
| Newly infected plants | 1.87% | 9.63% |
| Plants with increasing broom load | 2.24% | 11.67% |

Plants in the densely infected area of the field showed a higher incidence of new infections of witches' broom and increasing broom load than plants in the sparsely infected area. This suggests that disease spread is more rapid in areas of dense infection. Yet again, especially in the sparse population, the slow rate of disease spread from plant to plant, as well as within plants, is obvious.

### At a regional scale

Species affected by witches' broom?

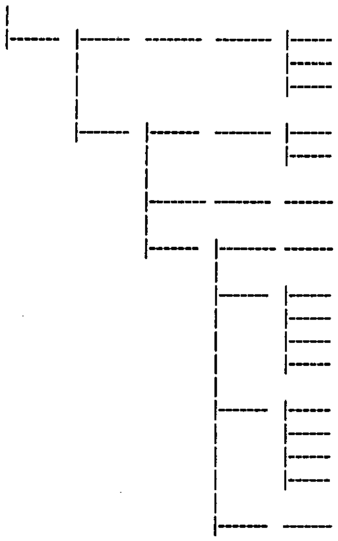
The extent of witches' broom infection can be expressed in the number of different species it affects. The presence of witches' broom in the genus *Protea* as well as the presence of witches' broom-like fasciation in other genera in the Proteaceae was compiled from published literature and data from atlasser sight record sheets (SRS's) from the Protea Atlas Project at UCT, dating back to 1992. The source of much of the information about species in the Proteaceae affected by witches' broom originates from SRS's filled in by atlassers of the Protea Atlas Project. The number of records for each species, and the number of these indicating the presence of witches' broom, or witches' broom-like fasciation, were noted.

*Results*: Witches' broom observed on *Protea* species is tabulated in Appendix 1. Witches' broom-like fasciation and miniaturization of vegetative shoots observed on species of genera other than *Protea* in the Proteaceae are listed in Appendix 2. A few specimens with fasciation on genera other than *Protea* were observed under a light microscope (including *Leucadendron salignum*, *Leucospermum hypophyllocarpodendron* and *Hakea sericea*), but there were no signs of the mite *A. proteae*. The number of parasite observations, indicated by the number of SRS's noting witches' broom, were included in both appendices. This data not only gives an idea of the extent of infection within a species, but also gives an indication of the robustness of the data, as a small likelihood exists that species misidentification or a misidentification of another growth as witches' broom may have taken place (for example, one record of infection was noted for *Protea repens*, yet both Dorrington (1988) and Rust (pers. comm.) have indicated that witches' broom has never been noticed on *P. repens*).

### Evolutionary pattern in the family or genus?

Cladograms constructed from gross morphological data of the Proteaceae family and *Protea* genus (Rebelo, unpublished) were used to investigate possible phylogenetic patterns of witches' broom infestation. The presence or absence of witches' broom was not included in the data used to construct the cladograms. The frequency of witches' broom within a species for the genus *Protea* is indicated on the cladograms. This was determined by dividing the number of records with witches' broom by the total records for each species.

*Results*: Cladograms of the genera in the Proteaceae and the species in the genus *Protea*, showing the distribution of witches' broom and witches' broom-like fasciation are shown in Figure 6 and Figure 7 respectively.

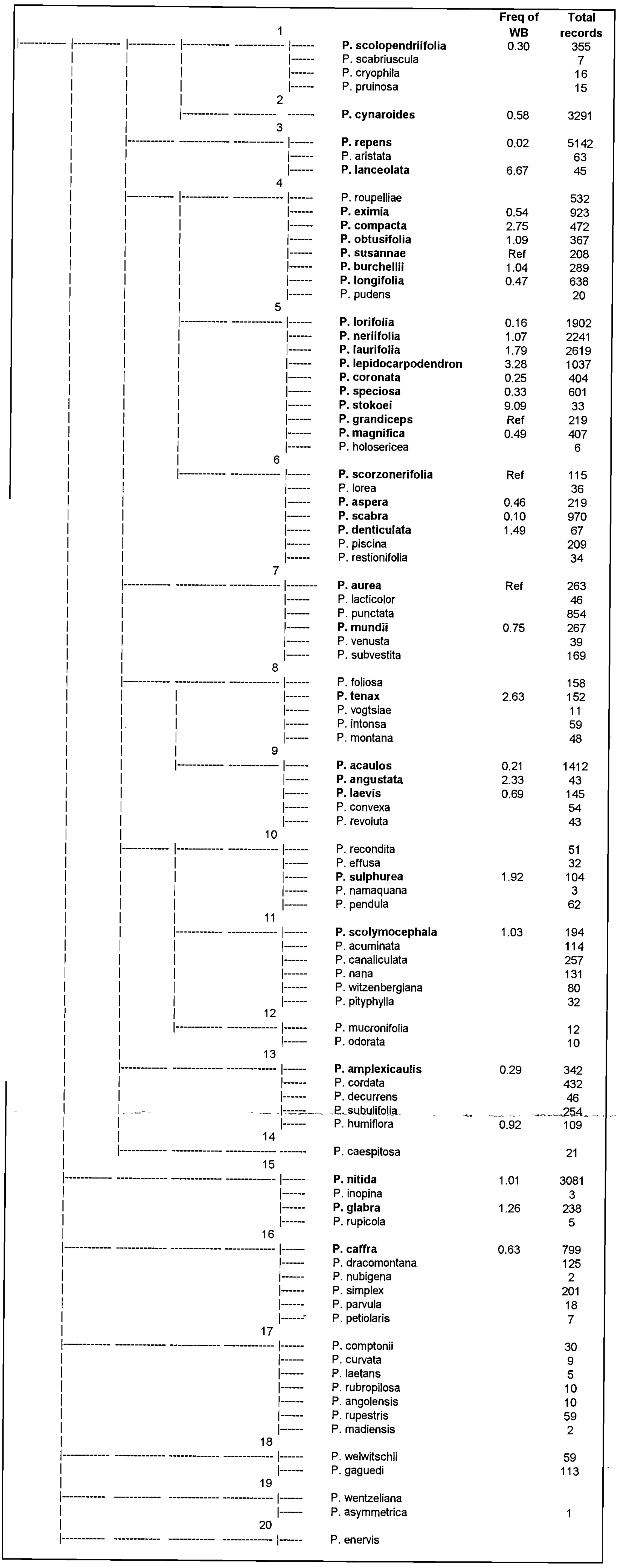


| | | No of species in genus | No of species with fasciation |
|---|---|---|---|
| Gr | Greyvillea | 2 | 0 |
| Br | Brabejum | 1 | 0 |
| Ha | **Hakea** | 4 | 1 |
| Pr | **Protea** | 91 | 33 |
| Fa | Faurea | 6 | 0 |
| Au | **Aulax** | 3 | 1 |
| Ve | **Vexatorella** | 4 | 1 |
| Ls | **Leucospermum** | 48 | 5 |
| Di | Diastella | 7 | 0 |
| Mi | Mimetes | 13 | 0 |
| Or | Orothamnus | 1 | 0 |
| Se | **Serruria** | 51 | 2 |
| Pa | Paranomus | 18 | 0 |
| So | Sorocephalus | 11 | 0 |
| Sp | Spatella | 20 | 0 |
| Ld | **Leucadendron** | 82 | 4 |

**Figure 6** A cladogram showing the evolutionary relationships between the genera of the Proteaceae in South Africa. The presence of fasciation within the genus is indicated in bold. The number of species within each genus, and the number of species affected by fasciation, are indicated on the cladogram.

The cladogram in figure 6 indicates that fasciation is present in a number of species in the Proteaceae. There is, however, no evolutionary restriction of fasciation to any part of the family. The cladogram in figure 7 indicates that witches' broom is found throughout the whole genus. Members of group 4 (Spoon-bract Proteas) and group 5 (Bearded Proteas) have almost all species affected by the disease.

Frequencies of witches' broom for each species have been included in the cladogram, as they give an indication of the prevalence of the disease within the species. Yet frequencies can be misleading, and should be viewed in conjunction with the total number of records.



|  |  | Freq of WB | Total records |
|---|---|---|---|
| 1 | **P. scolopendriifolia** | 0.30 | 355 |
|  | P. scabriuscula |  | 7 |
|  | P. cryophila |  | 16 |
|  | P. pruinosa |  | 15 |
| 2 | **P. cynaroides** | 0.58 | 3291 |
| 3 | **P. repens** | 0.02 | 5142 |
|  | P. aristata |  | 63 |
|  | **P. lanceolata** | 6.67 | 45 |
| 4 | P. roupelliae |  | 532 |
|  | **P. eximia** | 0.54 | 923 |
|  | **P. compacta** | 2.75 | 472 |
|  | **P. obtusifolia** | 1.09 | 367 |
|  | **P. susannae** | Ref | 208 |
|  | **P. burchellii** | 1.04 | 289 |
|  | **P. longifolia** | 0.47 | 638 |
|  | P. pudens |  | 20 |
| 5 | **P. lorifolia** | 0.16 | 1902 |
|  | **P. neriifolia** | 1.07 | 2241 |
|  | **P. laurifolia** | 1.79 | 2619 |
|  | **P. lepidocarpodendron** | 3.28 | 1037 |
|  | **P. coronata** | 0.25 | 404 |
|  | **P. speciosa** | 0.33 | 601 |
|  | **P. stokoei** | 9.09 | 33 |
|  | **P. grandiceps** | Ref | 219 |
|  | **P. magnifica** | 0.49 | 407 |
|  | P. holosericea |  | 6 |
| 6 | **P. scorzonerifolia** | Ref | 115 |
|  | P. lorea |  | 36 |
|  | **P. aspera** | 0.46 | 219 |
|  | **P. scabra** | 0.10 | 970 |
|  | **P. denticulata** | 1.49 | 67 |
|  | P. piscina |  | 209 |
|  | P. restionifolia |  | 34 |
| 7 | **P. aurea** | Ref | 263 |
|  | P. lacticolor |  | 46 |
|  | P. punctata |  | 854 |
|  | **P. mundii** | 0.75 | 267 |
|  | P. venusta |  | 39 |
|  | P. subvestita |  | 169 |
| 8 | P. foliosa |  | 158 |
|  | **P. tenax** | 2.63 | 152 |
|  | P. vogtsiae |  | 11 |
|  | P. intonsa |  | 59 |
|  | P. montana |  | 48 |
| 9 | **P. acaulos** | 0.21 | 1412 |
|  | **P. angustata** | 2.33 | 43 |
|  | **P. laevis** | 0.69 | 145 |
|  | P. convexa |  | 54 |
|  | P. revoluta |  | 43 |
| 10 | P. recondita |  | 51 |
|  | P. effusa |  | 32 |
|  | **P. sulphurea** | 1.92 | 104 |
|  | P. namaquana |  | 3 |
|  | P. pendula |  | 62 |
| 11 | **P. scolymocephala** | 1.03 | 194 |
|  | P. acuminata |  | 114 |
|  | P. canaliculata |  | 257 |
|  | P. nana |  | 131 |
|  | P. witzenbergiana |  | 80 |
|  | P. pityphylla |  | 32 |
| 12 | P. mucronifolia |  | 12 |
|  | P. odorata |  | 10 |
| 13 | **P. amplexicaulis** | 0.29 | 342 |
|  | P. cordata |  | 432 |
|  | P. decurrens |  | 46 |
|  | P. subulifolia |  | 254 |
|  | P. humiflora | 0.92 | 109 |
| 14 | P. caespitosa |  | 21 |
| 15 | **P. nitida** | 1.01 | 3081 |
|  | P. inopina |  | 3 |
|  | **P. glabra** | 1.26 | 238 |
|  | P. rupicola |  | 5 |
| 16 | **P. caffra** | 0.63 | 799 |
|  | P. dracomontana |  | 125 |
|  | P. nubigena |  | 2 |
|  | P. simplex |  | 201 |
|  | P. parvula |  | 18 |
|  | P. petiolaris |  | 7 |
| 17 | P. comptonii |  | 30 |
|  | P. curvata |  | 9 |
|  | P. laetans |  | 5 |
|  | P. rubropilosa |  | 10 |
|  | P. angolensis |  | 10 |
|  | P. rupestris |  | 59 |
|  | P. madiensis |  | 2 |
| 18 | P. welwitschii |  | 59 |
|  | P. gaguedi |  | 113 |
| 19 | P. wentzeliana |  |  |
|  | P. asymmetrica |  | 1 |
| 20 | P. enervis |  |  |

**Figure 7** A cladogram showing the evolutionary relationships between the species of the genus Protea. The presence of witches' broom within a species is indicated in bold. The recorded frequency of witches' broom, and total number of records of the species, is indicated to the right of the cladogram.

For example, if the total number of records for a species is low (say N < 50), then the chances of seeing witches' broom on that species would also be low, regardless of whether it is susceptible to the disease or not. Most of the species that have not been seen with infection do have low observation frequencies, and it may just be a matter of time before the disease is discovered on them. Alternatively, if the total number of records for a species is high (say N > 2000) and the frequency of witches' broom is very low (e.g. *P. repens*), it may indicate an error in either species identification or witches' broom identification.

## Discussion

Clearly the current methods of witches' broom removal from agricultural populations are ineffective, with one third of the plants from which the growth was removed in the agricultural population, reappearing at the same point 45 days after removal. This lack of success in effective disease eradication may be due to a poor understanding of the disease - and so assumptions about the disease processes which underlie the recommended phytosanitary methods will be discussed in light of the findings of the study below.

### Is the disease localized or systemic?

Although there is evidence that many plant viruses are transmitted from one generation to the next through seed (Johansen *et al.*, 1994), it does not appear from this study that that the probable causal agent (mycoplasma) can move in this way from the parent to the progeny. This statement is however based on the assumption that the seedlings from infected *P. cynaroides* adult plants will not develop witches' broom in the continued observation period of a year.

Both localized and systemic infection of plants by pathogens are documented for different diseases; these being two extremes of a possible continuum of host plant infection (Gäumann, 1950). Viral diseases, which are thought to be closely related to mycoplasmas, have been documented to produce both systemic (Gäumann, 1950) and localized (Smith, 1977) infections.

Evidence that witches' broom disease is localized within the plant is the eradication of the disease for a period of 100 days, from a prefire incidence of 0.6 brooms per plant at Jonkershoek site 2, after the fire severely burnt all plants and brooms. Further support that the disease is localized originates from the study at Jonkershoek site 1, in which the infection after the fire was not related to the infection before the fire (Table 3). One would expect that if the disease were systemic, there would be a relationship between the previous and current infection in resprouting plants.

Evidence that appears contradictory to the disease being localized is the aggregation of brooms within plants in Jonkershoek and Constantiaberg populations. Clumped broom distribution within a plant indicated infection on a plant was not due to chance alone, but that the presence of infection on a plant increases the risk of reinfection (Table 4). This infection patterning within a plant can be interpreted in a few ways, only one of which is that the disease might be systemic within the whole plant. Another interpretation just as likely, is reinfection within infected plants, should disease spread by mites occur on a small scale. A third interpretation is that it might indicate a differential susceptibility of plants to the disease. There would therefore be resistant plants with no infection, and susceptible plants with heavy infection.

The increased degree of clumping of brooms in *P. nitida*, indicated by a more highly significant Chi-squared value in *P. nitida* (resprouter) compared to *P. lepidocarpodendron* (reseeder), could also be viewed as evidence for systemic infection. However the lack of a relationship between previous (prefire) infection and current (postfire) infection in resprouting plants *P. nitida* shows this cannot be the case. The explanation for greater clumping and more infection in *P. nitida* compared with *P. lepidocarpodendron* might alternatively be due to small scale mite movement coupled with the larger size of *P. nitida* plants.

Additional evidence against the disease being localized, rather than systemic, is that of the poor success rate of parasite removal in agricultural plants (31% regrowth of witches' broom from areas of removal in 45 days). Having watched the methods of broom removal by workers, I have formulated a hypothesis as to why removal is ineffectual: that is because of an inability to identify and remove the disease from the point of infection, which is not the same as the point of symptom expression.

Removal of terminal or axillary brooms on aerial shoots in the *P. cynaroides* population was done by cutting the broom off the stem just below the broom. In some of the instances in which new brooms were observed, a few axillary infections had developed several centimeters just below the stem from which broom had been removed, indicating that the original infection point was possibly at the base of the whole stem, when the shoot was small, or a bud. All the vegetative growth above that infection point would then have contained the mycoplasma, and all buds would have the potential to develop into the observable symptom.

Certainly brooms that were removed from the base of the plant at the rootstock, and sometimes pulled off from the rootstock, quickly regrew. The infection point of these plants, especially if witches' broom was observed appearing in different places in the plant, was likely to have been at the young seedling stage, and all vegetative growth subsequent to that infection (i.e. the whole plant) would be infected and have the potential to express the symptom. Although this suggests that the disease is systemic, I would argue that it is not; rather that it is localized within the plant, but from the point of disease infection, rather than the point of symptom expression. Unfortunately these two points do not always coincide, and there is currently no simple way of determining the point of disease infection.

## Rates and patterns of disease spread

### Rates of disease spread

Disease spread in *Protea cynaroides* at Devon Valley, monitored over 45 days, was at a rate of 1.87% new infected plants per month in the area of the field that was sparsely infected (Table 7). In the densely infected area of the field, there was a rate of new infection of 9.63% plants per month (Table 7). The *P. nitida* population at Jonkershoek, monitored over 100 days, showed a rate of disease spread of 2.04% new infected plants per month. These rates disease spread are, especially in comparison with rates of disease spread in other pathogens, very slow. For example, rates of spread of *Phytophthora* on potatoes, can reach 100% foliage infection of an entire planted field in about 45 days (van der Plank, 1975).

### Patterns of disease spread

Disease spread appears to occur at two scales: 1) a local scale that encompasses the individual plant and near neighbours, and 2) long distance dispersal, enabling disease colonization of an infection-free population.

#### *Local patterns*

Possible evidence for short distance dispersal of the disease originated from individual and neighbour data, as well as spatial patterning in the natural Jonkershoek site 2 and agricultural population. Individual plant data in both natural populations revealed clumping of witches' broom within a plant (Table 4) - but whether this is indicative of local disease spread is not conclusive, as discussed above. Near neighbour studies, however, indicate in both the natural populations studied, that the infection on any particular plant is related to the infection of its immediate neighbours (Table 5 and Table 6). Constantiaberg data, in which the mean distance of neighbours to the target plant was 1.035 m ± 0.794 m, had a significant relationship of infection of neighbours with infection of target at $p < 0.05$. Jonkershoek data, in which the mean distance of neighbours was 5.042 m ± 3.592 m, had a near significant relationship between infection of neighbours and infection of target at $p < 0.1$.

The forward multiple stepwise regression model that was computed for Jonkershoek, included the distance of neighbours in the model. It is possible that the difference in the strength of relationship of the infection of the target plant with neighbour infection at Constantiaberg, compared to Jonkershoek, may be due to the difference in distance from nearest neighbours: the closer the neighbours, the stronger the relationship. In other words, at close range, neighbour infection would be the most important influence on target infection, but with bigger distances, the closeness of infected neighbours might be important as well. Unfortunately sample sizes are small, and so these ideas are mere speculation. Furthermore, this comparison has been drawn between two very different populations, in terms of species, plant age and different fire survival strategies. More evidence would thus be necessary for these ideas to be confirmed.

Assuming that infection at Jonkershoek site 2, in which broom was introduced, was due to the broom introduction; scales of witches' broom patterning can be seen in the appearance of the disease, 100 days after broom introduction (Figure 2). In this experiment, mite dispersal was a requirement for survival, rather than a means of dispersal, as brooms would be unable to support them once dried. It is interesting that only one of the trees on which brooms were tied was infected with witches' broom - the rest of the infections would be classed as near neighbour infections. It is possible that these mites were wind dispersed, although insectivorous birds might also visit brooms in search of food, and in this way disperse the mite. Direct evidence would be necessary to determine if one or both of these plausible dispersal modes are important in mite movement.

Lastly, patterning in *P. cynaroides* in the agricultural field at Devon Valley (Figure 4) indicated dense disease infection localized to plants in an area of the field approximately 25 m X 9 m. Outward spread of the disease from this area to the surrounding sparsely infected field did not occur, indicating disease spread to be highly local. The sparsely infected areas to either side of this densely infected area also showed very slow disease spread.

It is important to realize that all the conclusions drawn about disease spread assume similar rates of mite dispersal from one plant to the next, mycoplasma transferal from the mite into the plant, and symptom expression following infection (latent period). If this were not the case, the patterning of observed infection would be due to differential temporal disease expression, rather than spatial dispersal of the disease.

### *Long distance disease dispersal*

The long distance dispersal of the disease, which must exist in order for disease dispersal between isolated populations to occur, is still poorly understood. Long distance disease dispersal may occur as a single point infection from a distant source, or as a cloud from a distant source that may settle in an area, resulting in multiple infections (Pedgley, 1982 and Burdon, 1987). If, in the case of witches' broom, long distance infection was from a single point, whereafter disease spread was local, the disease would be expected to be very patchy as local spread is very slow.

Yet this does not agree with the description of the disease by farmers as "appearing suddenly and spreading like wildfire" through *Protea* plantations. The idea of a cloud of mites settling on a field, especially with local disease spread being so slow, far better fits the farmers' description of disease appearance and spread. Passive (and active) movement of insects in clouds by wind has well documented (Johnson, 1969 and Pedgley, 1982). Scale insects are carried by the wind in clouds (Pedgley, 1982), and it is likely that mites may be dispersed in the same way. Thus long distance dispersal of mites is probably *en masse* and by wind, as birds and humans would be unlikely to achieve the same magnitude of dispersal over long distances.

## Regional patterns

Witches' broom is common throughout the genus *Protea* (Figure 7). Many of the species on which the parasite has not been observed, have restricted distributions and few atlasser records, reducing the chance of sighting witches' broom. Yet some species, for example *P. repens*, are likely to be resistant to the disease.

Certainly the observation of two different species cultivated in a field alongside one another, with one species having heavy broom infection, whilst the other species is unaffected, is an interesting one. This suggests some degree of mite-host specialization, or at least a strong host preference by mites. Myburgh & Rust (1975) did suggest mite specificity, and these observations of disease patterning support their hypothesis.

### Recommended phytosanitary methods for agricultural populations

It follows from the discussion above that one of the assumptions about the disease was partially true, but the other assumption was false. A further observation of phytosanitary methods practised in the field has lead me to believe that there are three main reasons why following the procedures recommended by Rust (1984) and Coetzee *et al.*, (1989) has not lead to successful disease eradication.

The assumption of the disease being localized to the symptom expression of the disease, is partially true. However the witches' broom, which is the symptom expression, does not necessarily correspond with the actual disease infection, the point of which would most likely be lower than the lowest point of symptom expression. Removal of the symptom therefore would not automatically result in the removal of the disease. Advice to farmers would therefore be to include a large proportion of the stem below the witches' broom during removal, especially in *P. cynaroides*. Often infected branches are not removed at the base - probably because a potential flower-bearing stem is being removed, and one less flower translates into less profit. Plants that are heavily infected (for example *P. cynaroides* infection in the rootstock) should be removed and destroyed. Again, removal of infected plants (especially older plants) is expensive, as more young seedlings cost money, and even more importantly, time until flowering and profit realization may be substantially delayed.

The assumption of rapid disease spread within a field following an initial point source of infection is incorrect. This study has shown that the spread of the disease subsequent to the initial infection is very slow, and the scale of dispersal highly localized. The observation of farmers of witches' broom "appearing suddenly and spreading like wildfire" must therefore attributed to the other type of disease dispersal that is believed to exist in witches' broom - that of long distance dispersal. This type of dispersal is suggested take the form of a cloud of mites, possibly associated with certain meteorological conditions, that disperse by wind, and produce multiple infections on plants on their return to earth. Until the conditions that are conducive to this type of mite dispersal are known, it is virtually impossible to predict or advise farmers on what actions to take to avoid this type of infection, which is the most damaging one.

Another factor that I believe further weakens the effectiveness of disease removal from a field is that the removal guidelines are not adhered to strictly. During my studies I noted that staff working with broom removal did not rigourously follow the suggested guidelines. For example, on one occasion I observed that brooms that had been removed from plants in an agricultural population near Stellenbosch, had been thrown to one side of the field, and not removed and destroyed. Evidence from broom introduction to Jonkershoek site 2 (Figure 2), indicates that the disease can be spread from brooms removed from plants, especially if the mites are stressed into dispersing off of drying / dying brooms. Furthermore, mite dispersal due to irritability following insecticide exposure, resulted in them dropping off the plants and / or become aerial plankton (Rodriguez & Rodriguez, 1987). Removal guidelines are important, and should be carefully followed if any success in disease control is to be attained.

My impressions of the impact of witches' broom on flower farmers, is that it is considered more of a nuisance, rather than a threat to flower production. Although there is concern about witches' broom among the flower-farming community, most farmers do not suffer substantial losses because of the disease. Witches' broom also does not appear to be too debilitating to plants, with the exception of young infected seedlings.

Disease tolerance and control, strictly following the modified disease control guidelines, rather than attempts at disease eradication, may therefore at present be the best economical proposition for farmers. Suggested modified disease control guidelines would include:

1) Prevention of sale of infected plants in nurseries;
1) Mite control through regular broom removal rather than insecticide use;
2) Pruning of light infestations on adult plants in a sparsely infected area;
3) Removal of all plants in a densely infected area;
4) Removal of diseased seedlings; and,
5) Immediate burial or destruction of infected material by burning.

### Natural vs. agricultural studies

It is interesting to note that the results obtained from disease studies at Jonkershoek and Constantiaberg in natural populations supported the results that were obtained in the agricultural population at Devon Valley. None of the results lead to conflicting ideas on the functioning of the disease, despite these and other age and species differences. This highlights the value of studying natural plant populations in addition to directly studying agricultural populations, when studying problems, such as disease, in cultivated plants.

### Suggestions for future research

Suggestions for future research would include:

1) Experiments test the amount of stem removal below a broom necessary for disease eradication;
2) Experiments to test for the continued presence of mites (and thus potential for disease spread) in witches' brooms following successive broom removal;
3) Studies on the timing and eventual prediction the long distance dispersal of the mite, with reference to season and meteorological data. (It would be important to take the latent period between infection and symptom expression into account in these study);

4) Modelling of the financial costs of the removal of a single infected plant now compared with the slow spread of infection to neighbouring plants later. An equation could be formulated to indicate the point of infection density at which whole plant removal is more cost effective than repeated disease pruning;
5) Further research into temporal aspects of the disease, such as the variability of the disease latent period;
6) The analysis of infection occurrence and severity in plants with similar evolutionary histories, leaf phenologies (are broad-leaves more conducive to mite infection than narrow-leaves), flowering phenologies and habitat requirements;
7) Studies of plants in which disease resistance has been achieved in nature, and molecular research into the mechanism behind this resistance; and,
8) Studies involving the breeding of resistant species for flower farming.

## Acknowledgements

I would like to thank Prof. W.J. Bond for his supervision and thoughtful input; Dr. T. Rebelo for his with enthusiasm, guidance and assistance with the regional scale study; Ms E. Farnsworth for helpful comments on the report; Mr. J.R. Newton for support, and companionship and assistance in the field; Mr. S.W. Burger for computerizing the Jonkershoek site 2 map; Mr. P. van Rensburg for permission to study the disease at Etshwaleni and Cape Nature Conservation for permission to work on their property.

## References

Begon, M.; Harper, J.L. & Townsend, C.R. (1990). *Ecology: Individuals, populations and communities.* Second Edition. Blackwell Scientific Publications, Boston.

Borror, D.J.; Triplehorn, C.A. & Johnson, N.F. (1989). *An Introduction to the Study of Insects.* Sixth Edition. Harcourt Brace College Publishers, Fort Worth.

Boykin, L.S. & Campbell, W.V. (1984). Wind dispersal of the twospotted spider mite (Acari: Tetranychidae) in North Carolina peanut fields. *Environmental Entomology* **13**:221-227.

Burdon, J.J. (1987). *Diseases and Plant Population Biology.* Cambridge University Press, Cambridge.

Burdon, J.J.; Ericson, L. & Müller, W.J. (1995). Temporal and spatial changes in a metapopulation of the rust pathogen *Triphragmium ulmariae* and its host, *Filipendula ulmaria. Journal of Ecology* **83**:979-989.

Coetzee, J.H.; Rust, D.J. & Latsky, L.M. (1986). Mites (Acari) on proteas. *Acta Horticulturae* **185**:247-248.

Coetzee, J.H.; van Broembsen, S.L. & Brits, G.J. (1989). Sanitation of proteas to control pests and diseases. *The SA Nurseryman* **8**(2):28-29.

Dorrington, R. (1988). Witches' broom disease of *Protea cynaroides. SAPPEX News* **59**:20.

Forsberg, L. (1993). Protea diseases and their control. *The Queensland Agricultural Journal Reprint* p.1-13.

Gäumann, E. (1950). *Principles of Plant Infection.* Crosby Lockwood & Son, Ltd. London.

Hoy, M.A.; van de Baan, H.E.; Groot, J.J.R. & Field, R.P. (1984). Aerial movements of mites in almonds: Implications for pest management. *Calif. Agric.* **38**(9):21-23.

Johansen, E.; Edwards, M.C. and Hampton, R.O. (1994). Seed transmission of viruses: current perspectives. *Annu. Rev. Phytopathol.* **32**:363-386.

Johnson, C.G. (1969). *Migration and Dispersal of Insects by Flight.* Methuen & Co Ltd, London.

Knox-Davies, P.S.; van Wyk, P.S. & Marasas, W.F.O. (1988). Regional reviews of diseases in Proteaceae: South Africa. *Journal of the International Protea Association* **16**:50-55.

Manners, J.G. (1982). *Principles of Plant Pathology.* Cambridge University Press, Cambridge.

Meynhardt, J.T. (1986). Proteas: pests and diseases. *Flowers, Ornamental Shrubs and Trees Series Reprint* **B6**:1-4.

Myburgh, A.C. & Rust, D. (1971). Witches broom of proteas. *Information Bulletin* **34**. Fruit and Food Technology Research Institute, Stellenbosch. 3p.

Myburgh, A.C. & Rust, D. (1975). A survey of the pests of the Proteaceae in the western and southern Cape Province. *J. ent. Soc. sth. Afr.* **38**(1):55-60.

Myburgh, A.C.; Rust, D.J. & Starke, L.C. (1973). Pests of protea cut-flowers. *J. ent. Soc. sth. Afr.* **36**(2):251-255.

Pedgley, D.E. (1982). *Windborne Pests and Diseases: Meteorology of Airborne Organisms.* Ellis Horwood Limited, Chichester.

Rebelo, T. (1996). Worldmap and the Protea Atlas Project. *Plantlife* **15**:26-32.

Rodriguez, J.G. & Rodriguez, L.D. (1987). Nutritional ecology of phytophagous mites. In: F. Slansky Jr. & J.G. Rodriguez (Eds). *Nutritional Ecology of Insects, Mites, Spiders and Related Invertebrates.* John Wiley and Sons, New York.

Rust, D. (1984). Witches broom. *SAPPEX News* **42**:15-16.

Rust, D. & Myburgh, A.C. (1965). "Heksebesem" in proteas. . *Indigenous flowers* **5**. Fruit and Food Technology Research Institute, Stellenbosch. 3p.

Rust, D.J. & Myburgh, A.C. (1976). Heksebesem by proteas. *Veld & Flora* **62**:21-22.

Smith, K.M. (1977). *Plant Viruses.* Sixth Edition. Chapman and Hall, London.

Turner, M.G. 1989. Landscape ecology: the effect of pattern on process. *Ann. Rev. Ecol. Syst.* **20**:171-197.

van Broembsen, S.L. (1989). *Handbook of Diseases of Cut-Flower Proteas.* International Protea Association, Monbulk, Victoria, Australia.

Van der Plank, J.E. (1975). *Principles of Plant Infection.* Academic Press Inc., New York.

# Appendix 1

Alphabetical list of all *Protea* species known to be affected by witches' broom. The information source is acknowledged, from the Protea Atlas Project, by SRS. The number of records of witches' broom (No. WB) per species obtained from the Protea Atlas Project are included.

| Species | Information source | No. WB |
|---|---|---|
| *P. acaulos* | SRS | 3 |
| *P. amplexicaulis* | SRS; Rust & Myburgh (1965) | 1 |
| *P. angustata* | SRS | 1 |
| *P. aspera* | SRS | 1 |
| *P. aurea* | Pers. comm. (Rust) | |
| *P. burchellii* | SRS; Rust & Myburgh (1965) | 3 |
| *P. caffra* | SRS; Rust & Myburgh (1965) | 5 |
| *P. compacta* | SRS; Rust & Myburgh (1965) | 13 |
| *P. coronata* | SRS; Rust & Myburgh (1965) | 1 |
| *P. cynaroides* | SRS; Rust & Myburgh (1965) | 19 |
| *P. denticulata* | SRS | 1 |
| *P. eximia* | SRS; Rust & Myburgh (1965) | 5 |
| *P. glabra* | SRS; Rust & Myburgh (1965) | 3 |
| *P. grandiceps* | Rust & Myburgh (1965) | |
| *P. humiflora* | SRS; Rust & Myburgh (1965) | 1 |
| *P. laevis* | SRS | 1 |
| *P. lanceolata* | SRS | 3 |
| *P. laurifolia* | SRS; Rust & Myburgh (1965) | 47 |
| *P. lepidocarpodendron* | SRS; Rust & Myburgh (1965) | 34 |
| *P. longifolia* | SRS; Rust & Myburgh (1965) | 3 |
| *P. lorifolia* | SRS; Rust & Myburgh (1965) | 3 |
| *P. magnifica* | SRS; Rust & Myburgh (1965) | 2 |
| *P. mundii* | SRS | 2 |
| *P. neriifolia* | SRS; Rust & Myburgh (1965) | 24 |
| *P. nitida* | SRS; Rust & Myburgh (1965) | 31 |
| *P. obtusifolia* | SRS; Rust & Myburgh (1965) | 4 |
| *P. repens* | SRS | 1 |
| *P. scabra* | SRS | 1 |
| *P. scolopendriifolia* | SRS | 1 |
| *P. scolymocephala* | SRS | 2 |
| *P. scorzonerifolia* | Rust & Myburgh (1965) | |
| *P. speciosa* | SRS | 2 |
| *P. stokoei* | SRS | 3 |
| *P. sulphurea* | SRS; Rust & Myburgh (1965) | 2 |
| *P. susannae* | Pers. comm. (Atlasser) | |
| *P. tenax* | SRS | 4 |

## Appendix 2

Species other than *Protea* of the Proteaceae that have been found with witches' broom-like fasciation. The information was obtained from the SRS records of the Protea Atlas Project. The number of records of witches' broom (No. WB) obtained from the Protea Atlas Project are included.

| Species | No. WB |
|---|---|
| *Aulax umbellata* | 2 |
| *Hakea sericea* | 2 |
| *Leucadendron gandogeri* | 1 |
| *Leucadendron procerum* | 1 |
| *Leucadendron salignum* | 8 |
| *Leucadendron stellare* | 1 |
| *Leucadendron teretifolium* | 2 |
| *Leucospermum arenarium* | 1 |
| *Leucospermum conocarpodendron* | 1 |
| *Leucospermum hypophyllocarpodendron* | 2 |
| *Leucospermum parile* | 1 |
| *Leucospermum praemorsum* | 1 |
| *Serruria furcellata* | 1 |
| *Serruria phylicoides* | 1 |
| *Vexatorella obtusata* | 2 |