Development of a diagnostic test for the pathogens which cause cavity spot of carrot

Conducted on behalf of
Horticulture Research International
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For the Horticultural Development Council

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Project Title: Development of a diagnostic test for the pathogens which cause cavity spot of carrot

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Location of project: HRI Wellesbourne

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APPLICATION

The objective of the project was to produce a diagnostic process to give growers information on the likely risk of cavity spot caused by *Pythium violae* or *Pythium sulcatum*. The process developed involved collection and processing of soil from fields possibly scheduled to be cropped with carrots followed by the conduct of a competition ELISA. Absorbance ratios were interpreted to indicate degrees of risk of disease based on extensive field testing. Within the time scale of the project detection of the common *P. violae* was well refined; detection of the rarer *P. sulcatum* was less certain. Following a period of pilot-scale commercial application, the process has been commercialised with Oxford Agricultural Consultants.

SUMMARY

The objective of the project was to develop a practical, serology-based assay for the pathogens which cause cavity spot of carrot. Because of considerations which growers make when selecting fields to crop with carrots, the assay was required to produce a risk assessment for a particular field before the grower was committed to drill the seed.

Over a three year period, extensive field work demonstrated that the major cavity spot pathogen (*Pythium violae*) could be detected in soil with polyclonal antisera in a competition ELISA test. Levels of detection, measured as absorbance ratios derived from concentrated and non-concentrated soil extract samples, were highly correlated with the cavity spot which subsequently developed on crops. These results gave a good guide to our grower collaborators on necessary fungicide usage, and demonstrated the potential both for appropriate treatment of 'at risk' crops, and for cost savings by identifying fields where benefit was unlikely to accrue from the application of fungicide.

With experience, it was found that fields where absorbance ratios were less than 1.5 would generally produce carrots with less than 5 % of carrots with cavities. As ratios increased, risk also increased, and where values were consistently in the area of 2 - 3, the crop required treatment with metalaxyl at the recommended rate. Values which were higher, up to the theoretical maximum of 17.5, indicated high risk of disease, suggesting that the field should not be cropped with carrots in that season.

Results showed that in late spring and summer as soils dried out, absorbance ratios universally fell to the baseline. Experience has shown that fields which produce heavily infected crops in one year may not do so in subsequent years. This is taken to mean that the signal measured by this assay reflects actively growing fungus; when the soil dries the fungus loses viability, and may not leave significant inoculum to infect the next crop. It is therefore essential to determine activity of the pathogens in the period immediately before sowing is planned.

Growers have the possibility of reducing disease risk by the choice of cultivars with some field resistance. From HDC funded work at Stockbridge House it has been shown that cultivars such as Nandor produce crops with considerably less disease than would be the case with more susceptible types. Appendix Tables 1 and 3 indicate those cultivars currently considered to have field resistance. Manipulation of sowing and harvest dates may also be employed to the same end. Where late sowing is possible, the grower may benefit from drilling after soils have partially dried and activity of the pathogen has declined. Monitoring crops on a regular basis may enable the grower to apply fungicide to a part-grown crop should cavity spot begin to develop, further, early harvesting may be used to prevent significant loss in more mature crops.

For *Pythium sulcatum*, the other causal agent of cavity spot, only one outbreak occurred in the fields surveyed and that was not predicted by the absorbance ratios. From laboratory studies it

is known that the antiserum against *P sulcatum* works in the competition assay when infested soil is processed. At present we do not have a basis for interpreting data obtained for *P sulcatum* in the field, other than to suggest that where absorbance ratios are very high the field should not be cropped with carrot.

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EXPERIMENTAL SECTION

Introduction

Cavity spot is the major disease of carrot in Western Europe and other temperate areas where the crop is grown. The disease is caused primarily by the slow-growing fungus *Pythium violae*, with a small number of outbreaks resulting from infection by *Pythium sulcatum* (White, 1984; 1988). The disease caused by the former species may be controlled by costly application of a fungicide containing metalaxyl at high rates. Cost and environmental considerations make prophylactic spraying of carrot crops undesirable. *P. sulcatum* is not controlled by metalaxyl, so applications where that fungus is causal would not be expected to control the disease. In extensive survey work in the eastern counties (White, 1988) it was found that many applications of the fungicide were made to crops where there was no significant disease, and therefore the crop would not benefit from the application. Against this background, HDC decided to fund the present project to develop a method whereby the degree of activity of the fungi in soil might be assessed to provide a guide to the likely risk of cavity spot in a field.

At the commencement of the project, monoclonal antibodies (MAbs) were just begining to be made available as diagnostic reagents for fungi. Their benefits, as compared to polyclonal antisera (PAbs) were considered to be higher specificity and unlimited production for subsequent commercialisation. Initially therefore, an early objective was to raise MAbs to *P. violae* and *P. sulcatum*, but considerable difficulty was experienced and those produced did not exhibit the necessary specificity. The effort was therefore switched to raising PAbs against a range of potential fungal antigens. The later part of the project considers the use of these PAbs in extensive field survey work which led to pilot-scale, and finally fully commercial scale assays.

Materials and Methods

Fungal cultures and antigen preparation

Fungi were isolated from commercial carrots from the major cropping areas in the UK using a medium of corn meal agar amended with rifamycin and pimaricin (White, 1988). These were identified to species level and added to the HRI Culture Collection. Selected isolates of *P. violae* and *P. sulcatum* were used for antigen preparation by transfer through 1.5% water agar and inoculation into 200 ml modified Petri medium (White, Lyons, Wakeham, Mead and Green, 1994). When cultures had produced sufficient growth, mycelium was removed and blotted dry. Samples were then ground in liquid nitrogen to yield a fine powder

following evaporation of the nitrogen. The powder was re-suspended in phosphate buffered saline (PBS) at 1.5-2 ml/g of mycelium, the resulting suspension was filtered through cotton gauze and centrifuged at 45000 g at 4°C for 30 min. The supernatant and pellet were both retained and stored at -20°C until use.

Production of MAbs and PAbs

Two MAbs and three PAbs were raised against *P. violae* and *P. sulcatum* (Table 1), the former being produced in the first two years of the project in collaboration with Dr Jon Green at The University of Birmingham. Later, further PAbs were produced from the same organisms.

Table 1 Types and origins of immunological reagents

Monoclonal antibodies	Immunogen
UB 13	*P. violae*-cell wall/membrane
UB 14	*P. sulcatum*-cell wall/membrane
Polyclonal antisera	
88/6	*P. sulcatum*-cell wall/membrane
88/7	*P. violae*-cell wall/membrane
RPC1	*P. violae*-cell wall/membrane
92/39	*P. sulcatum*-cytoplasmic fraction
92/41	*P. violae*-cytoplasmic fraction

MAbs were raised in BALB/c mice which were immunised intraperitoneally with 0.2 ml of phosphate buffered saline (PBS) containing 50 µg protein from one of the two fungi. After similar immunisations approximately 5 and 8 weeks later, fusions were performed with the NSO myeloma cell line using the methods described by Galfre & Milstein (1981) and Estrada-Garcia *et. al.* (1989). After 10-14 days, tissue culture supernatants from growing hybridomas were tested in ELISA and two cell lines producing MAbs designated UB13 (raised against *P. violae*) and UB14 (raised against *P. sulcatum*) were cloned by limiting dilution. MAbs UB13 and UB14 were subsequently used only in basic work on serological profiling of the genus *Pythium* (White, *et al*, 1994).

PAbs were prepared in New Zealand White female rabbits using intramuscular injections of antigen in combination with Freund's incomplete adjuvant. The initial PAbs were raised using cell wall/membrane extracts of the two fungi standardised at 250 µg protein/ml with sterile physiological saline. Initial injections used complete adjuvant, while incomplete adjuvant was used for booster injections which were at one week intervals for six weeks. Serum was separated and stored with the addition of 0.02% sodium azide in 0.5 ml aliquots at -20°C. The later PAbs were produced using the supernatant cytoplasmic phase of the cell wall extracts.

Development of the competition ELISA system

It was known that detection levels in indirect ELISA tests were limited to ± 0.5-1 µg protein/ml, which would not be sufficiently sensitive for this work, and it was therefore necessary to consider more sensitive assays. The competition or inhibition ELISA makes use of a step where the test sample is reacted with the PAb before the latter is used against standardised homologous (= self) antigen in a solid-phase assay. Any recognition between the antigen in the sample and the PAb results in formation of a complex and reduction of immunoglobulin levels in the assay. When compared with unreacted PAb this is shown by a reduction of signal in the solid-phase stage. This form of assay is expected to give good recognition of antigen at < 100 ng /protein/ml (5-10 times more sensitive than indirect ELISA).

Soil extraction procedure

Soil samples were stored until use at 4°C. Moisture content was determined by heating 80 g fresh soil in an oven at 90°C for 48 h to enable the calculation to give 50 g equivalent dry soil for the assay. The appropriate amount for each soil sample was weighed into a 100 ml Ehrlenmeyer flask to which was added 50 ml of 0.02% sodium azide solution. The flasks were sealed with Clingfilm and then shaken vigorously on an orbital shaker for 24 h, after which the flasks were left to stand for 24 h to allow sedimentation of the slurry. The aqueous layer was then decanted

into universal bottles and centrifuged at 950 g for 30 min. The supernatant was filtered through Whatman No 1 qualitative filter paper into 30 ml Sarstedt screw capped plastic containers. From each of these, 15 ml was removed and put into a similar container. All samples were frozen and stored at -20°C, before those comprising the 15 ml sub-samples were freeze dried. The original sample extract and its freeze dried sub-sample were paired and stored at -20°C until required for the ELISA assay. To prepare for ELISA, samples were removed from the freezer and thawed. The freeze dried samples were resuspended in 750 µl of 0.02 % sodium azide solution. The test as applied to cavity spot tissue is described in detail by Lyons & White (1992). Following ELISA, the ratio for absorbance between the original sample and the concentrated sub-sample was in each case calculated by dividing the former by the latter. In the later stages of the work this process was integrated with the Vax microcomputer and the output from the ELISA plate reader was exported to Genstat which in turn produced absorbance ratios for all samples in relation to position on each plate.

Tests with naturally infested field soils

Soils from three fields with a known history of cavity spot were compared with soil from a field where no disease had been recorded. Two of the cavity spot positive fields had been shown to have disease caused by *P violae*, the other with *P sulcatum*. All three cavity spot fields gave a positive reaction with the *P violae* antibody (88/7), but there was little signal from the

non-cavity spot field. The *P sulcatum* antibody (88/6) did not give a positive reaction with soil from any field. In other work, with artificially inoculated soil, this PAb gave high signals, so it must be assumed that *P sulcatum* was not active in the field soil.

At this stage in the project HDC reqiured the work to be taken to extensive field sampling to determine the potential value of the process to growers.

Extensive field testing of the competition ELISA system

In 1991, plots were established in a large number of fields at Ingham, Suffolk (Haywards Foods) and around Gooderstone, Norfolk (W H Knights)(Table 2). The plots were placed in opposite corners of the fields and in the centre of 25 m squares which were prominently marked to prevent spraying with fungicide containing metalaxyl. Each plot comprised two beds width x 6 m length of row, and was split into two halves. At the first visit, 450 g samples of soil were removed from each half of each plot, giving four samples for each field in the exercise. For each sample the pH was determined, and a sub-sample of 50 g was removed for processing through the competition ELISA procedure using antibodies 88/6 and RPC 1. Data for pH was used in correlation calculations with the final percentage cavity spot values for each plot. From each soil sample, a further sub-sample was carefully air-dried and the number of colony forming units (cfu) of the mycoparasite *Pythium oligandrum*/g soil was determined by

the method of White, Wakeham & Petch (1992). Correlations were calculated between these values and those for final percentage cavity spot.

At different times following establishment of the crops, visits were made to recover carrots and further soil samples from each field. The carrots were washed and assessed for cavity spot by the method described by White (1988) in which three size grades of cavities were counted separately. This system slightly overestimates the number of infected roots as compared with commercial assessments, since the smaller lesions would not result in rejection at grading. The soil samples were sub-sampled as described above and processed by competition ELISA.

In 1992, the formal field experimentation was limited to just ten fields around Gooderstone which were managed as described above. Cavities were counted in two size grades, following the decision to amalgamate the smaller size grades. The initial sampling of the fields was conducted as part of a pilot commercial scale operation, with in principle eight soil samples being removed on standard sampling patterns from a large number of fields (number of samples taken by the grower was varied according to field size), and the ten fields were then selected for this study. The antisera used in ELISA were the cytoplasmic PAb's 92/39 and 92/41.

Soil pH, Pythium oligandrum *and cropping histories*

For every soil sample taken in the two years, readings were made

of soil pH (10 g soil in 25 ml distilled water), colony forming units of the natural mycoparasite *Pythium oligandrum* using the method described by White (1992). Previous cropping histories were obtained, where possible, from the co-operating growers.

Results

Field data, soil associations and carrot cultivars for 1991 are shown in Appendix Table 1, with the cropping histories which could be obtained in Appendix Table 2. The equivalent information for 1992 is contained in Appendix Tables 3 and 4. The absorbance ratios for *P violae* and *P sulcatum* for all fields in 1991 are shown respectively in Figs 1 and 2. Those for the 1992 fields are shown in Table 2. Sampling of carrots was done at regular intervals from July 1991 through to February 1992 in the first survey year, and from June 1992 to February 1993. The results are considered separately for the two years of the survey.

1991

The most obvious features of the ELISA data for *P violae* are some high absorbance ratios in fields 1-10, with ratios > 14 for field 8. Values thereafter were 2 or less. Data for *P sulcatum* followed a similar pattern, although the maxima were < 7. At the first sampling of Knights fields, carrots were taken from all fields and the data for percentage cavity spot are shown in Fig 3. Sampling of Haywards processing carrots was not appropriate because they were too small. While 26 of the 37 fields at Knights

had < 10 % of roots with any cavities and many were in the range 1-2 %, a small number showed c. 20 % disease, and in field 8 70.5 % of the crop had lesions. As indicated above, recording cavities in the 1.0 mm size range causes an overestimate of the commercially significant cavities. At this sampling, this equated to a mean of 2.5 % of lesions across all fields, so for many fields percentage infection was effectively zero.

It was established at this stage that *P violae* was the causal agent of cavity spot in field 8 and most other fields. In field 1, disease was the result of infection by *P sulcatum*. By this time, the absorbance ratios for both fungi had fallen to 2 or less in most fields.

A smaller scale sampling was made in August, when Haywards fields were included. The plots in field 32 could not be located because of high, dense foliage, but values for cavity spot and absorbance ratio were low in the remaining four fields.

In September, for 20 of the 31 fields, percentage cavity spot was less than 10, with values indicating a small increase compared with the July recordings. Cavity spot in field 1 had increased to 41.0 % and in field 8 to 88.0 % (Fig 4). In the former, the disease was not controlled in the remainder of the crop which had been sprayed with metalaxyl and mancozeb, and the grower had abandoned the field. In the latter, disease had been held back by the fungicide spray, and the field was subsequently strawed in October and the crop harvested around the end of the year. Absorbance ratios remained consistently low on all fields. Data missing from Fig 4 reflected normal commercial harvesting of the

Fig.1 Absorbance ratios for *Pythium violae*, for all fields in the 1991 survey derived from soil samples collected in April

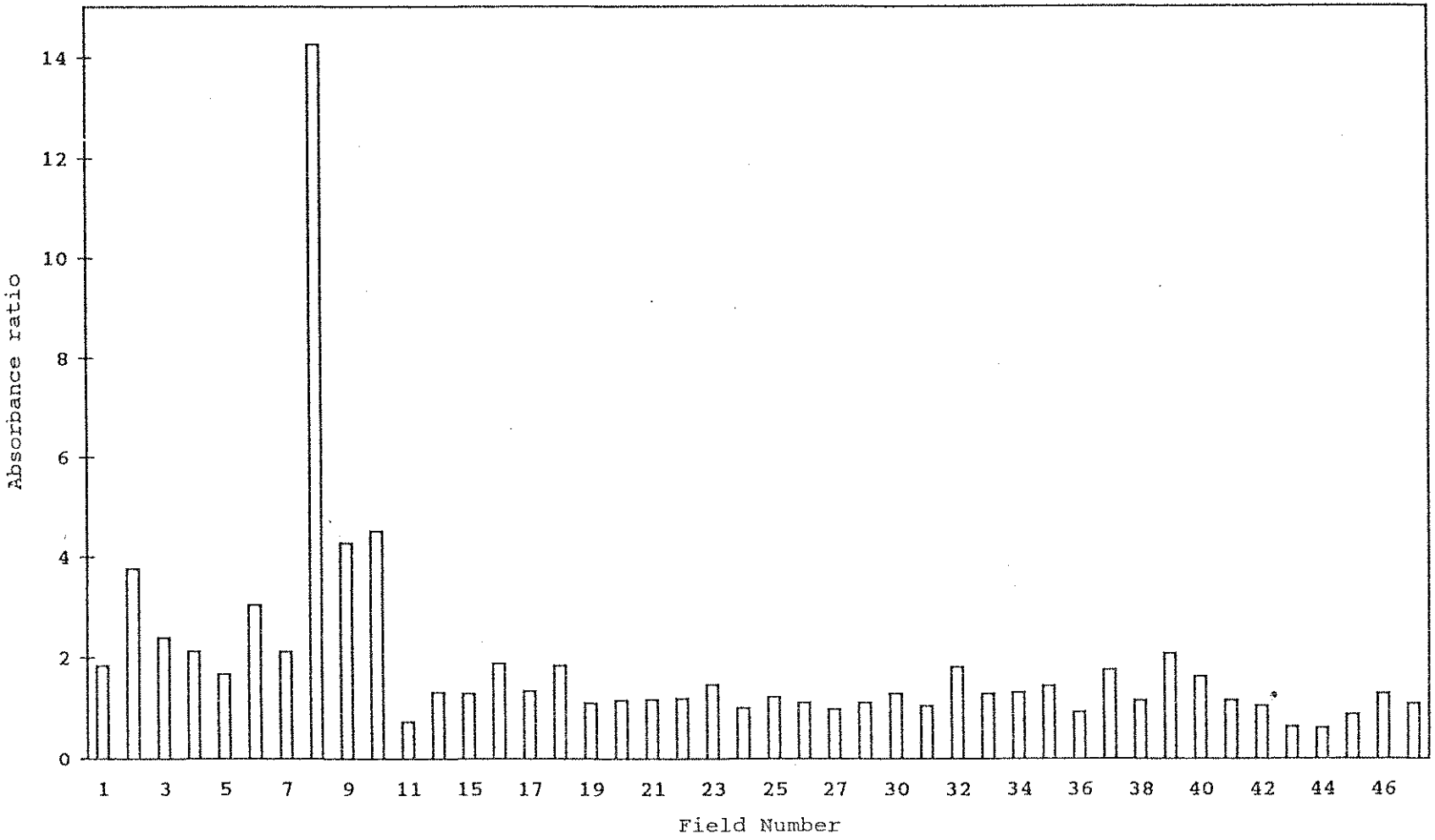


Fig.2 Absorbance ratios for *Pythium sulcatum*, for all fields in the 1991 survey derived from soil samples collected in April

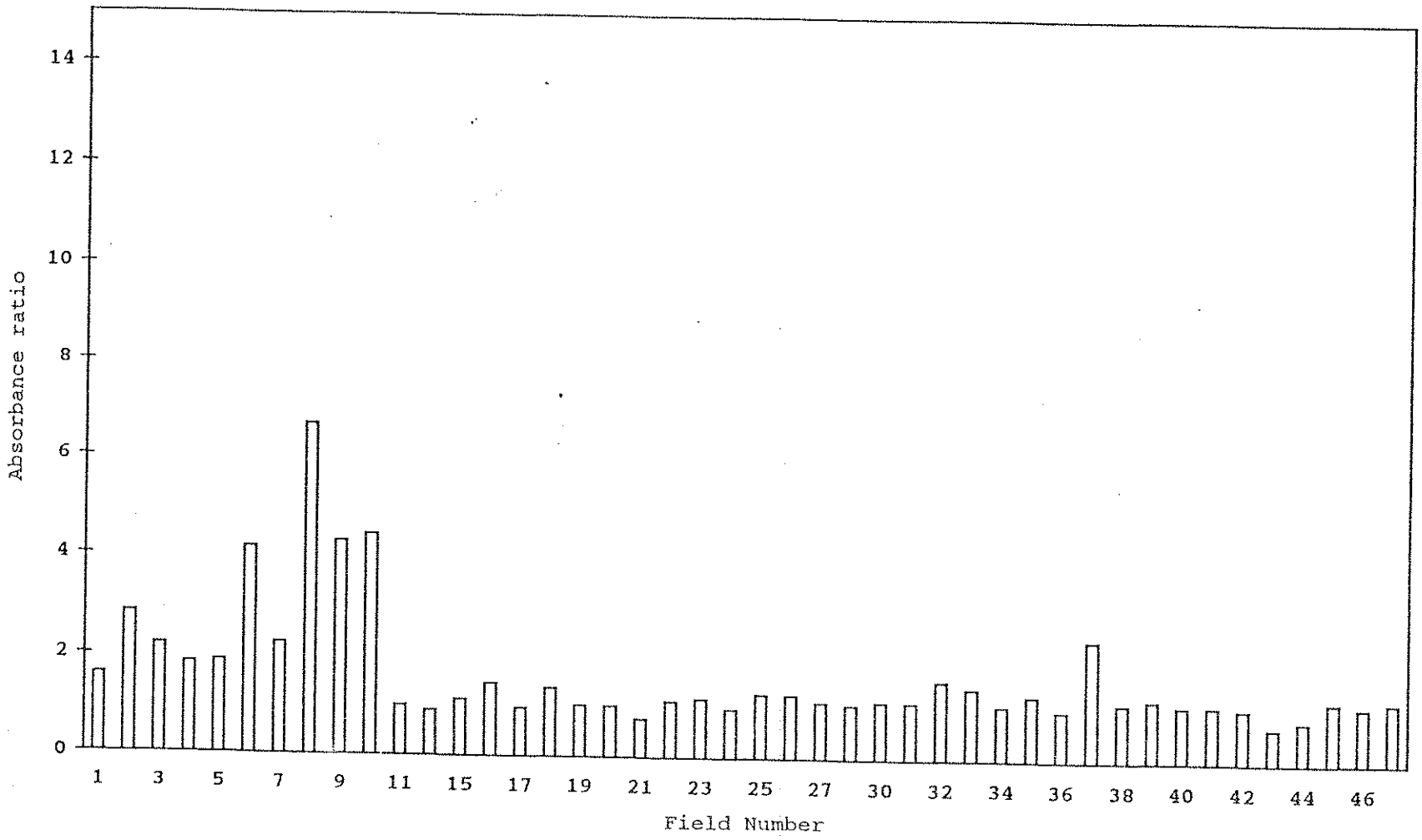
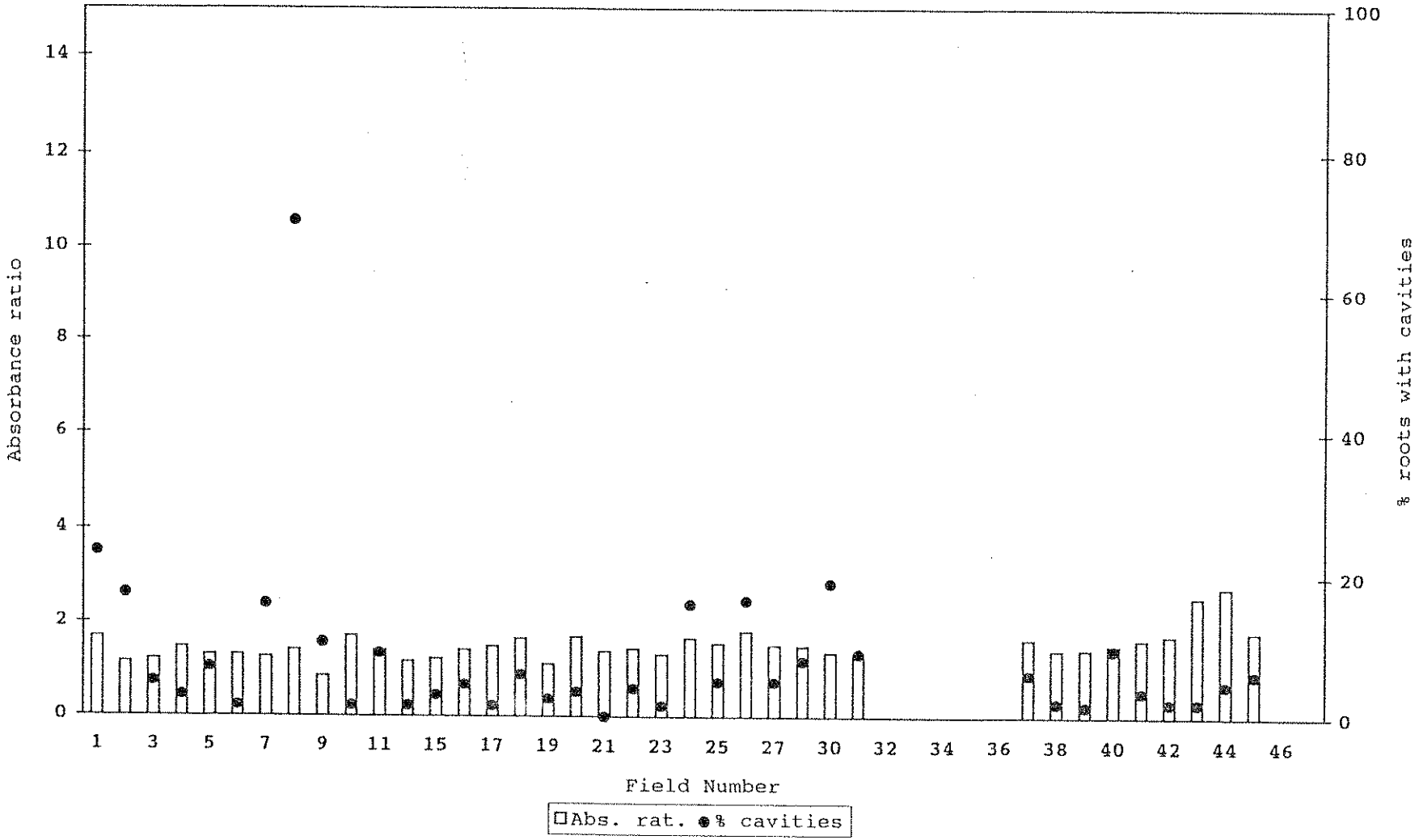


Fig.3 Absorbance ratios for *Pythium violae*, plus percentage cavity spot, for Knights fields in the 1991 survey based on soil and carrots collected in July



fields which necessarily included our plots because of the cultivation and future cropping demands of the grower.

The final major sampling was in October, before strawing, when all remaining Knights fields and four of Haywards were included (Fig 5). The data are substantially the same as is described above, with further increase in disease in fields 1 and 8. In 22 of the 31 fields less than 10 % of carrots had cavity spot. Mean absorbance ratios were again consistently low.

By December 1991 there were only 10 of the Knights fields remaining (Fig 6) and of those, two still had less than 10% disease. Seven of those were harvested before the January sampling, and in February, only field 24 remained. During this period, there was no increase in percentage disease in any field, although severity (number of lesions per root) of disease continued to increase on carrots in field 8 throughout the sampling period. Further, no lesions in the smallest category were recorded, indicating increase in lesion size with time. For field 24, which was 110 acres, the final percentage of carrots with cavity spot in our survey was 14.0. The grower harvested the whole field with what was for a crop so late in the season relatively insignificant cavity spot. Figs 7 and 8 illustrate the contrast between cavity spot development following high absorbance ratios in field 8 and a mean absorbance ratio of 1.0 in field 24.

Fig.4 Absorbance ratios for *Pythium violae*, plus percentage cavity spot, for Knights fields in the 1991 survey based on soil and carrots collected in September

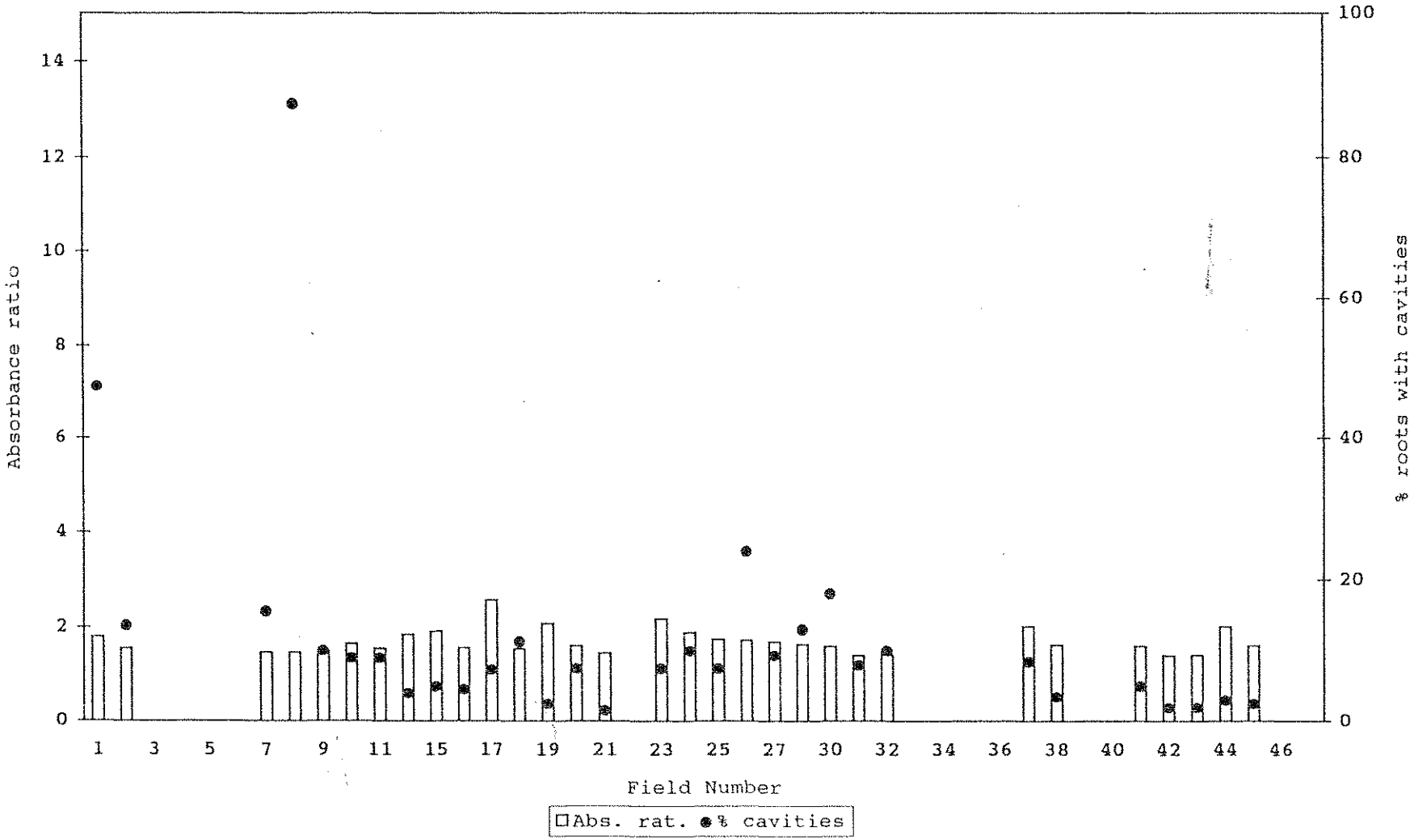


Fig.5 Absorbance ratios for *Pythium violae*, plus percentage cavity spot, for Knights fields in the 1991 survey based on soil and carrots collected in October

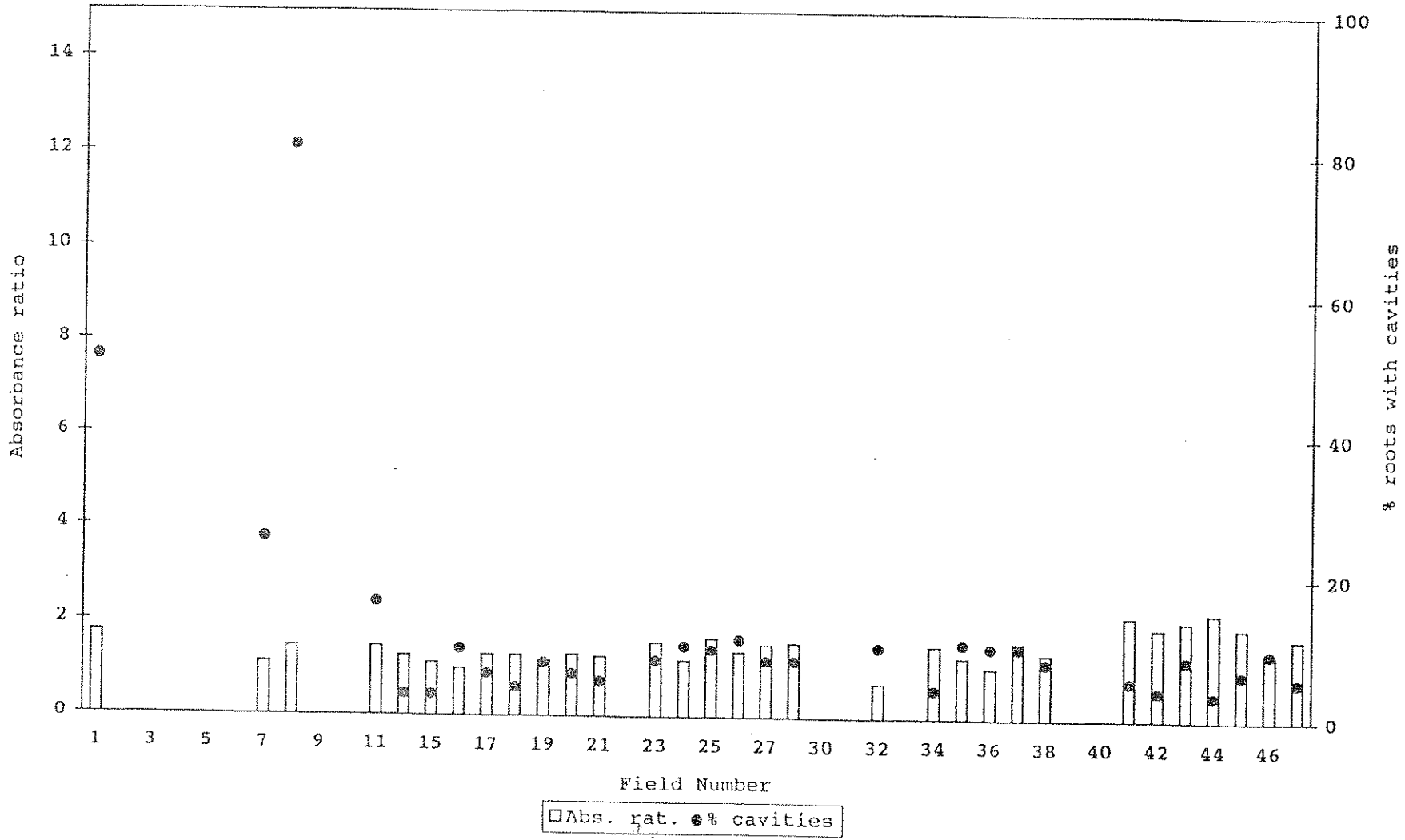
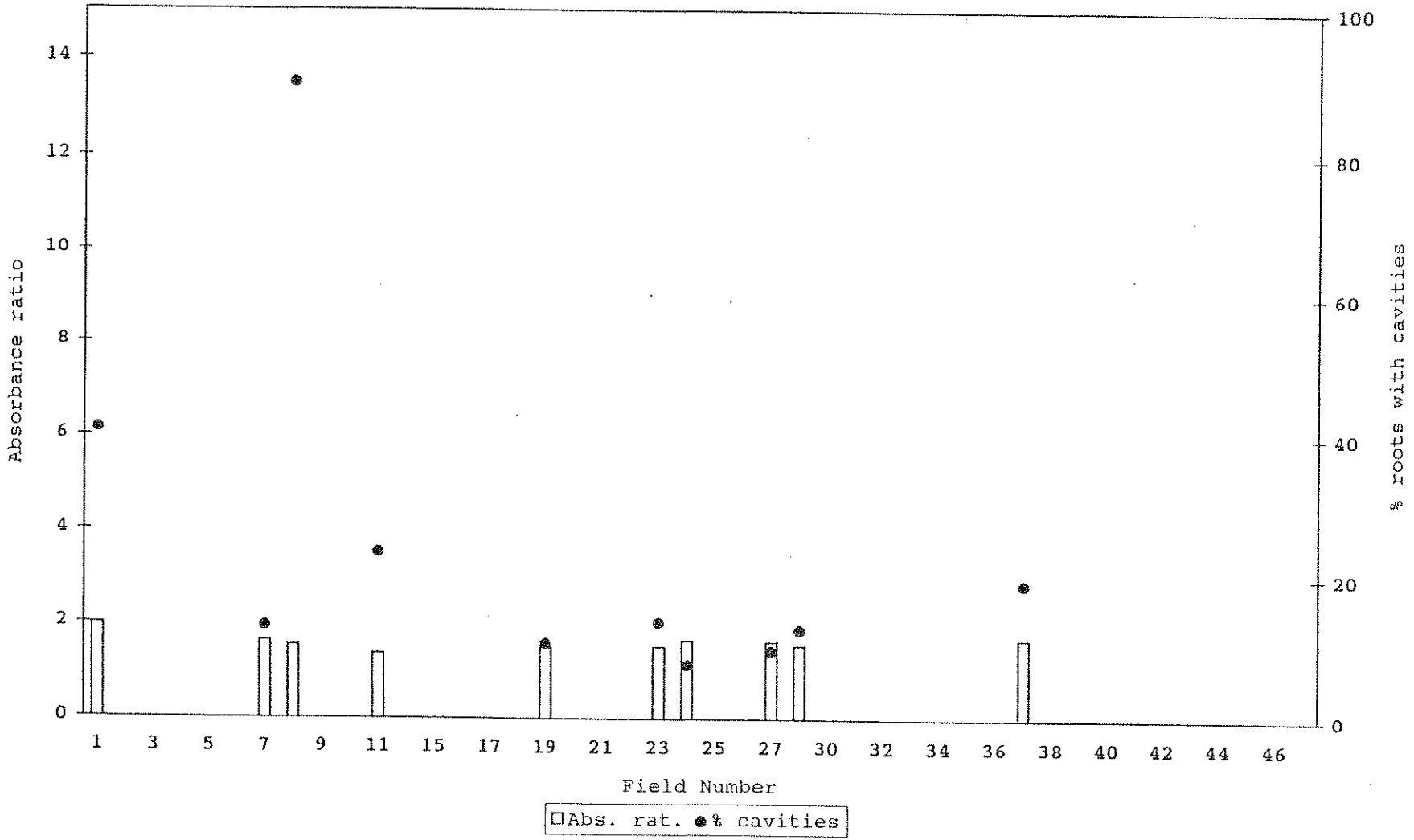


Fig.6 Absorbance ratios for *Pythium violae*, plus percentage cavity spot, for Knights fields in the 1991 survey based on soil and carrots collected in December



The nature of the data produced is most suitable for statistical analysis by the use of contingency tables. Those tables summarising the above data are shown overleaf. For both *P violae* and *P sulcatum* there was a highly significant correlation ($P < 0.001$) between the original absorbance ratios obtained in April, and percentage cavity spot.

As it is now known that only in field 1 was the disease caused by *P sulcatum*, the second correlation appears eccentric. This is discussed later in the report.

The summary of the above data is that for the majority of fields where there were low absorbance ratios, percentage cavity spot was subsequently low. In most fields the general level of disease increased slowly between July and December. While the major outbreak of disease in field 8 was predictable, there was no indication that the outbreak of *P sulcatum*-based cavity spot would occur. The relatively high absorbance ratios in some of the fields 1-10 did not necessarily result in high disease, partly because some of the fields were harvested early.

Table 2. Year 1 Contingency tables, all absorbance values against all cavity spot results.

Contingency table *P. violae*

Absorbance ratio		% cavity spot			
		Below 5	5 - 10	10 - 15	Above 15
Below 0.99		5	4	6	6
1.00 - 1.49		83	51	46	40
1.50 - 1.99		69	31	34	42
Above 2.00		28	16	14	10

Pearson chi-square value = 7.21 with 9 df p = 0.615

(p 0.05 = 16.919)

Contingency table *P. sulcatum*

Absorbance ratio		% cavity spot			
		Below 5	5 - 10	10 - 15	Above 15
Below 0.99		9	6	6	3
1.00 - 1.49		124	59	66	55
1.50 - 1.99		37	30	25	32
Above 2.00		15	7	3	8

Pearson chi-square value = 10.52 with 9 df p = 0.310

(p 0.05 = 16.919)

Fig.7 Field 8 - 1991 absorbance ratios and cavity spot development

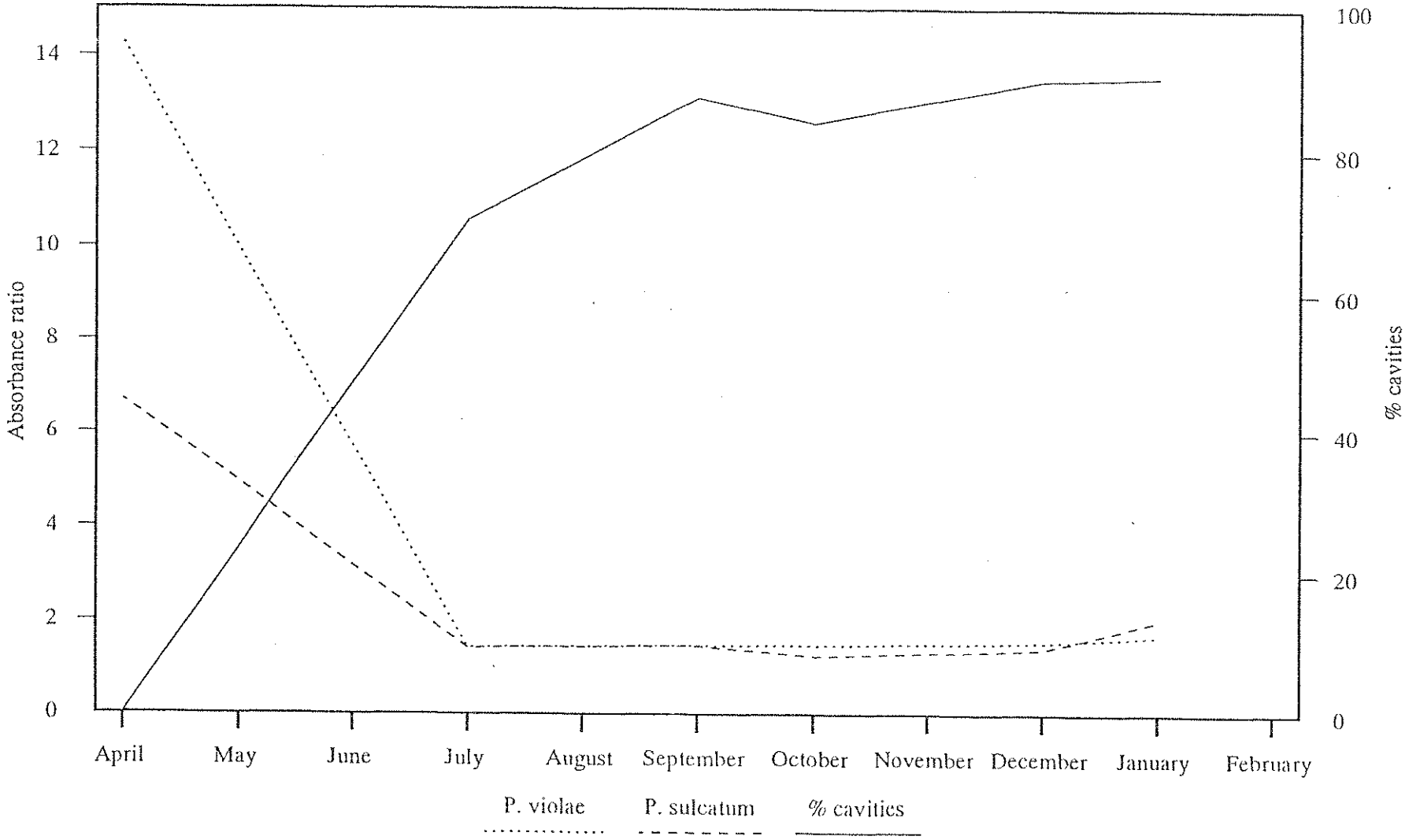
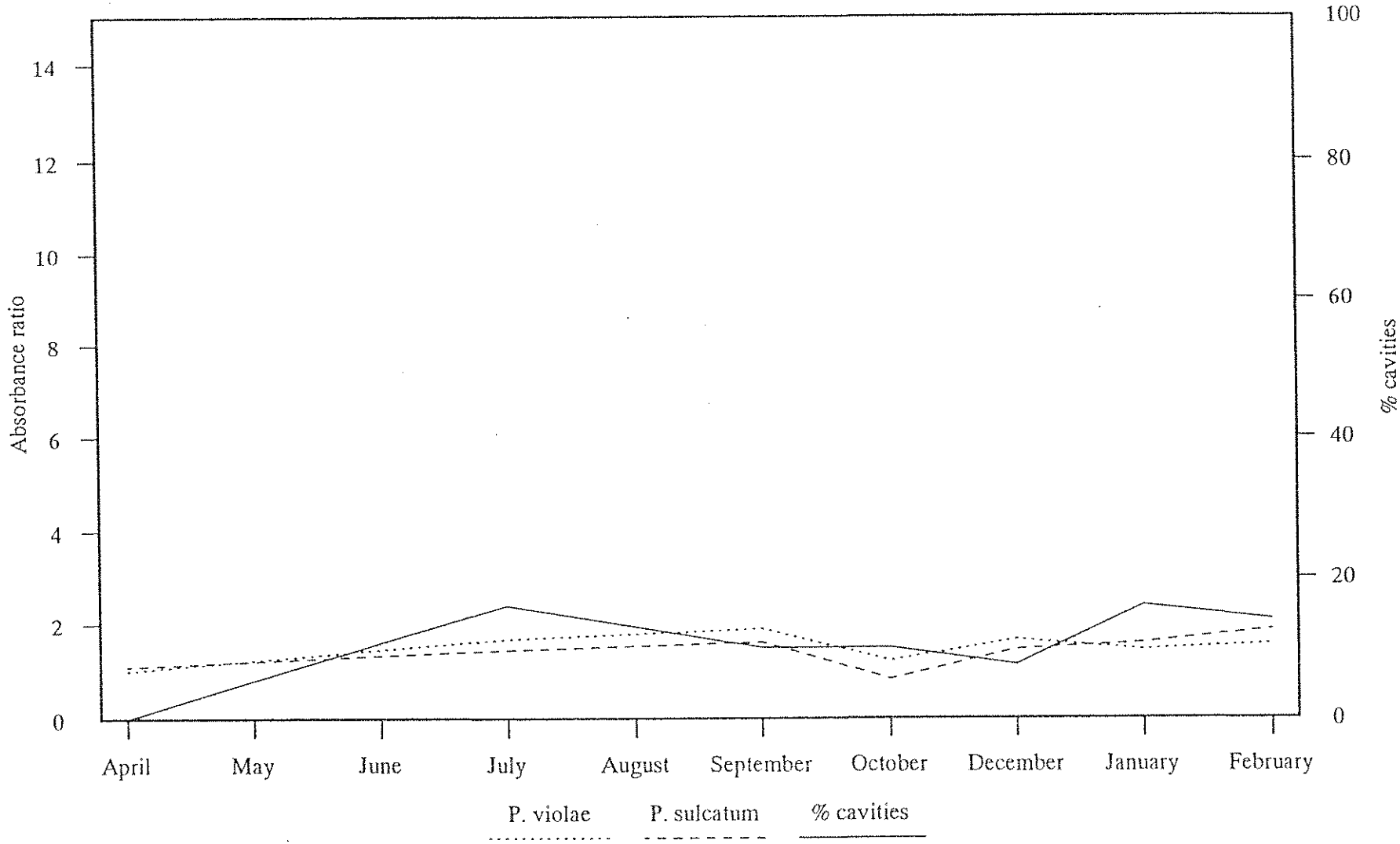


Fig.8 Field 24 - 1991 absorbance ratios and cavity spot development



1992

The absorbance ratios obtained in the commercial sampling for both species of *Pythium* from the ten fields chosen for detailed study are shown in Table 3. Commercial decisions made on The Entry (Field 2), Parkers L Shaped (Field 5) and River Meadow (Field 8) were to drill late and harvest early.

At the first full sampling in June (Fig 9), absorbance values were mainly in the range 2 - 4 both for *P violae* and *P sulcatum*. Carrots of a size suitable for harvesting were available in two fields, although there was little cavity spot, values being 3 % or lower.

By July, although percentage cavity spot in nine of the ten fields was low, that in field 2 was 26.5 % (Fig 10). Absorbance ratios remained in the range 2-4.

It was confirmed that all fields with disease had cavity spot caused by *P violae*.

By September (Fig 11), the disease in seven of the ten fields remained < 3 %, two fields had been harvested and in Field 2, 27.2 % of carrots had lesions. Absorbance ratios had fallen considerably compared with those in earlier months.

At subsequent harvests in October and November (Figs 12 and 13), the results remained substantially the same, although one more

field had been harvested. Cavity spot in field 2 was at 65.9 % in October, and 80.3 % in November. The field had by this time been harvested without significant disease and our area was not strawed. As a result, by the time of the February visit (Fig 14), the roots had decayed to such an extent that the true level of cavity spot could not be determined. For the three remaining fields, disease levels remained low.

Because of the relatively small scale of the above exercise, the data are not suitable for statistical analysis.

Table 3 Absorbance ratios for *Pythium violae* and *Pythium sulcatum* from soil samples assayed commercially in the winter of 1991

Pythium violae

Field	No. of samples	Range of absorbance ratios	Mean absorbance ratio
1. Janes Wood	9	1.40-2.67	1.96
2. The Entry	11	1.30-4.86	2.28
3. Wilkins Heath	21	0.92-2.06	1.46
4. Heygate	11	1.50-2.47	2.00
5. Parkers L Shaped	7	1.82-4.86	2.55
6. 13 acre	5	1.43-2.19	1.85
7. Bone Mill	7	1.56-3.00	1.97
8. River Meadow	5	2.17-3.35	2.62
9. Grange	5	1.54-2.49	1.93
10. Top Camp	7	1.29-3.48	1.91

Pythium sulcatum

1. Janes Wood	9	1.39-3.29	2.12
2. The Entry	11	1.35-3.29	1.87
3. Wilkins Heath	21	1.01-2.55	1.55
4. Heygate	11	1.60-3.69	2.10
5. Parkers L Shaped	7	1.94-6.76	2.94
6. 13 acre	5	1.32-2.45	2.10
7. Bone Mill	6	1.41-2.33	1.91
8. River Meadow	5	1.82-3.73	2.85
9. Grange	5	1.36-2.42	1.75
10. Top Camp	7	1.24-1.58	1.42

Fig.9 Absorbance ratios for *Pythium violae*, plus percentage cavity spot, for Knights fields in the 1992 survey based on soil and carrots collected in June

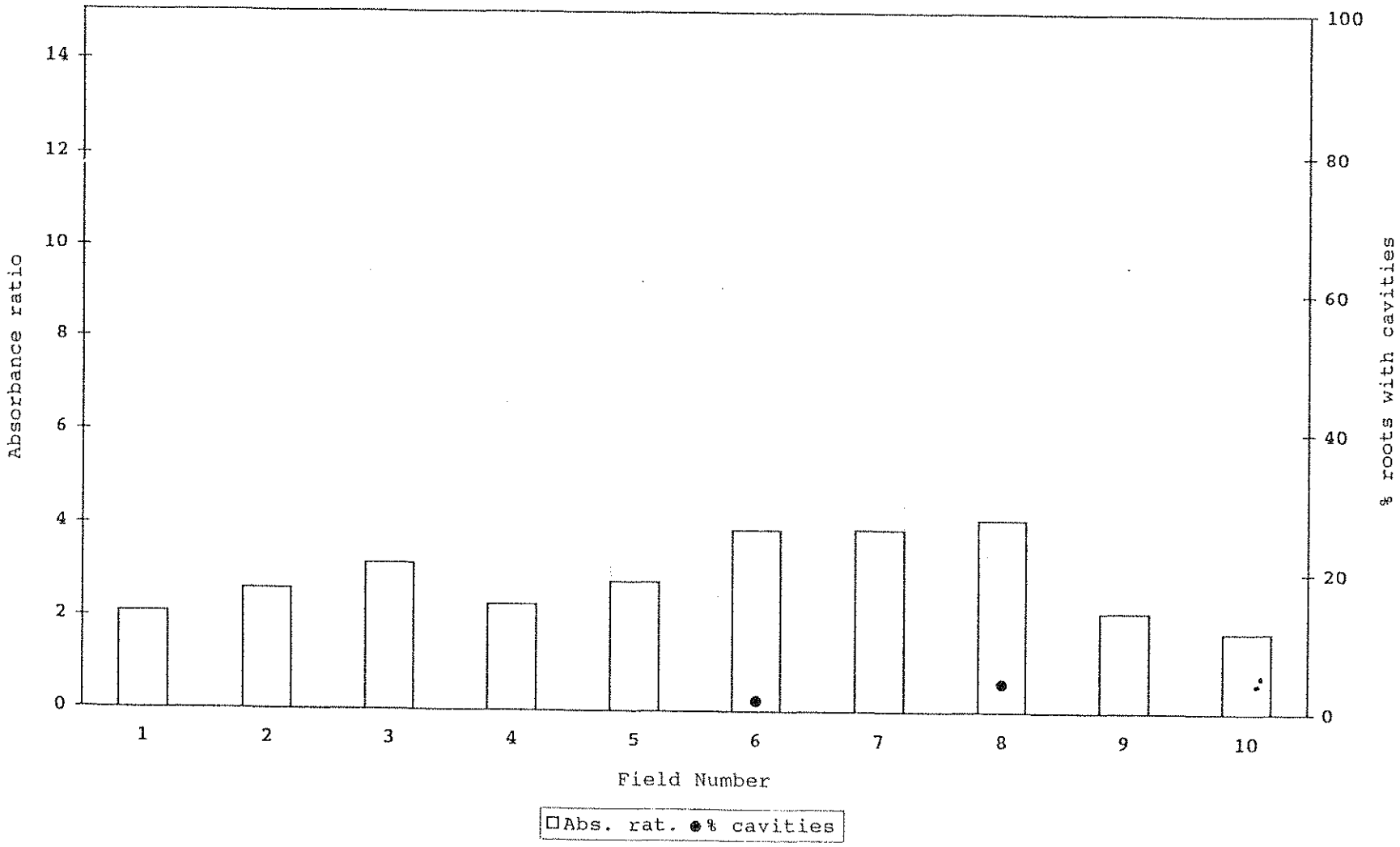


Fig.10 Absorbance ratios for *Pythium violae*, plus percentage cavity spot, for Knights fields in the 1992 survey based on soil and carrots collected in July

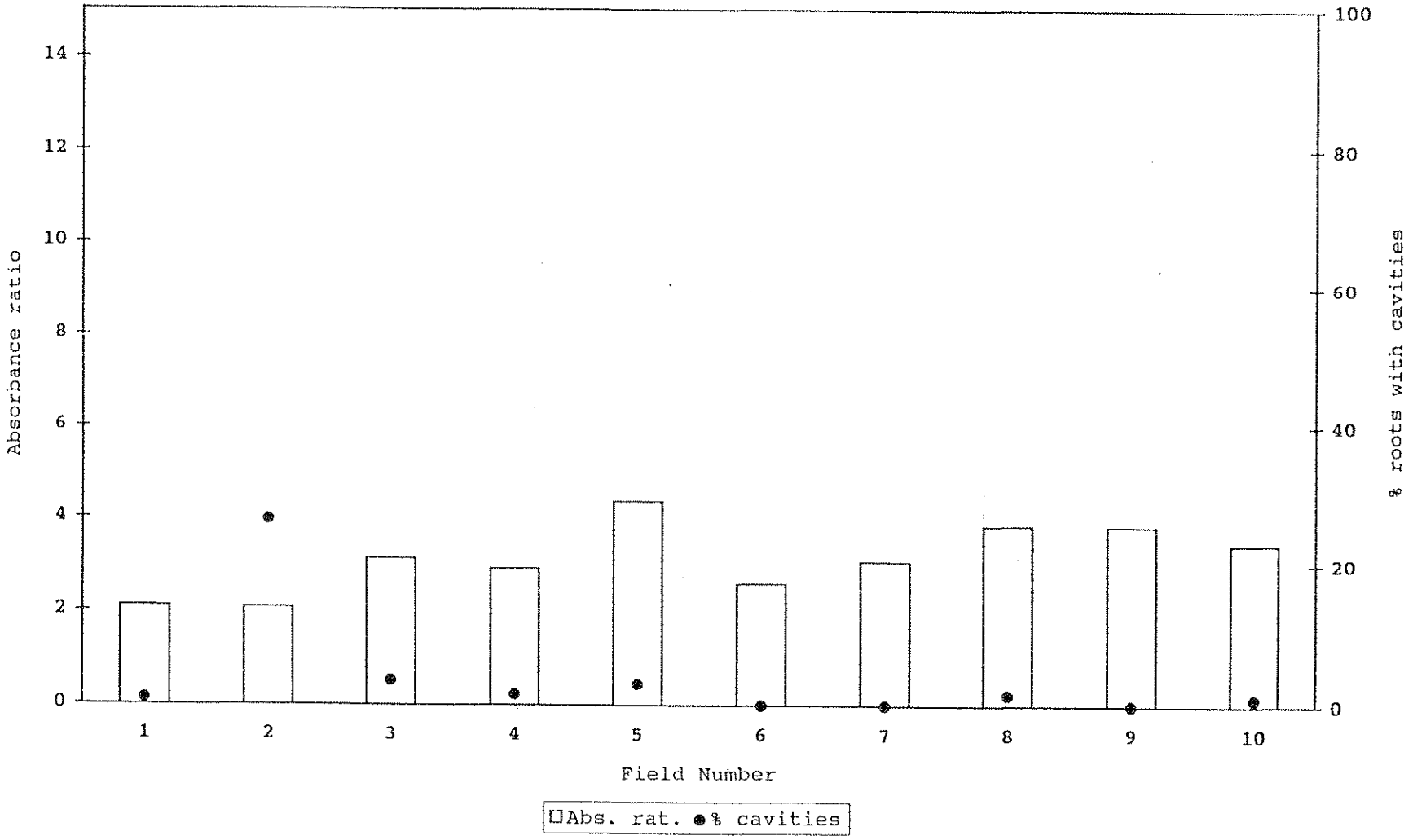


Fig.11 Absorbance ratios for *Pythium violae*, plus percentage cavity spot, for Knights fields in the 1992 survey based on soil and carrots collected in September

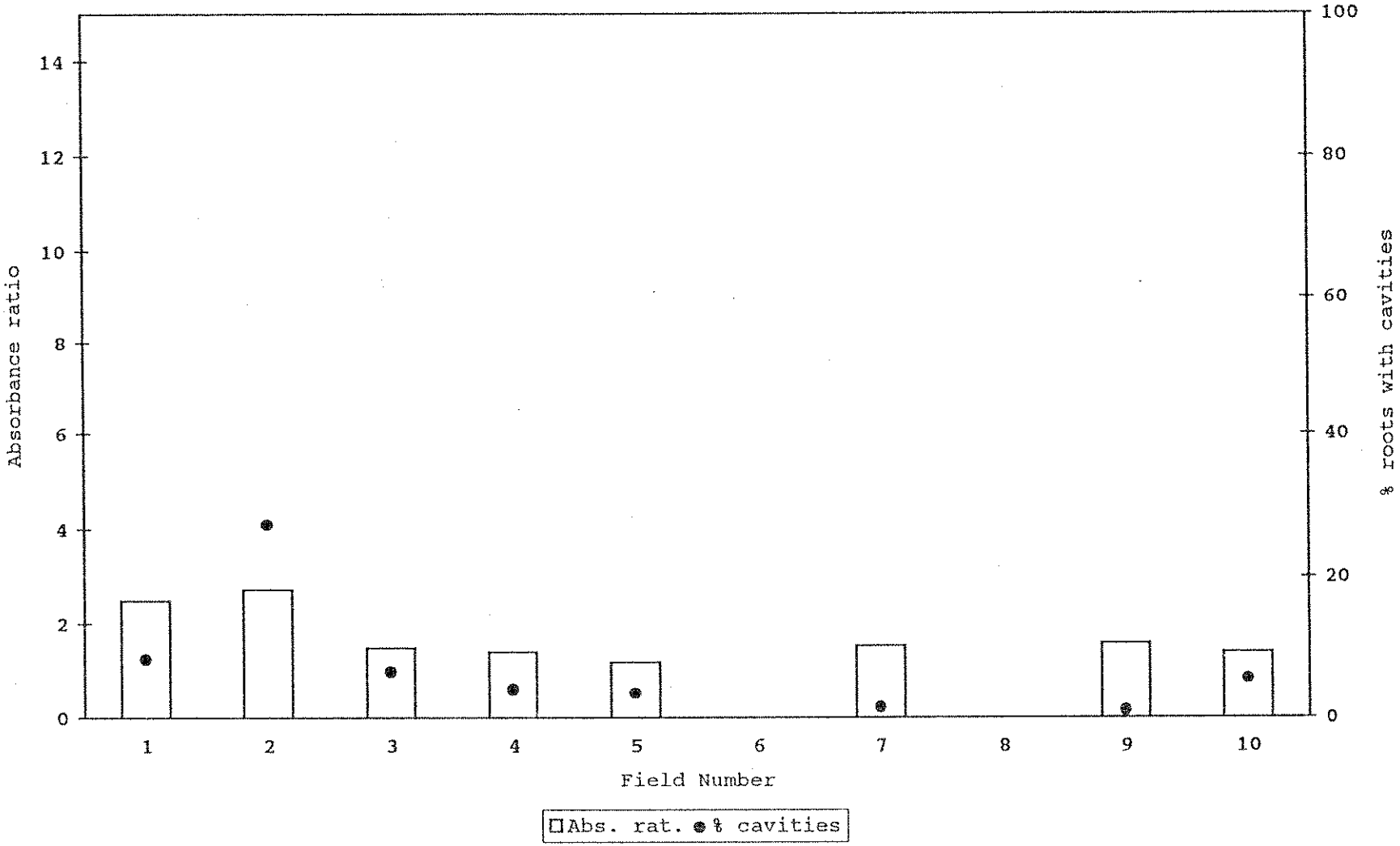


Fig.12 Absorbance ratios for *Pythium violae*, plus percentage cavity spot, for Knights fields in the 1992 survey based on soil and carrots collected in October

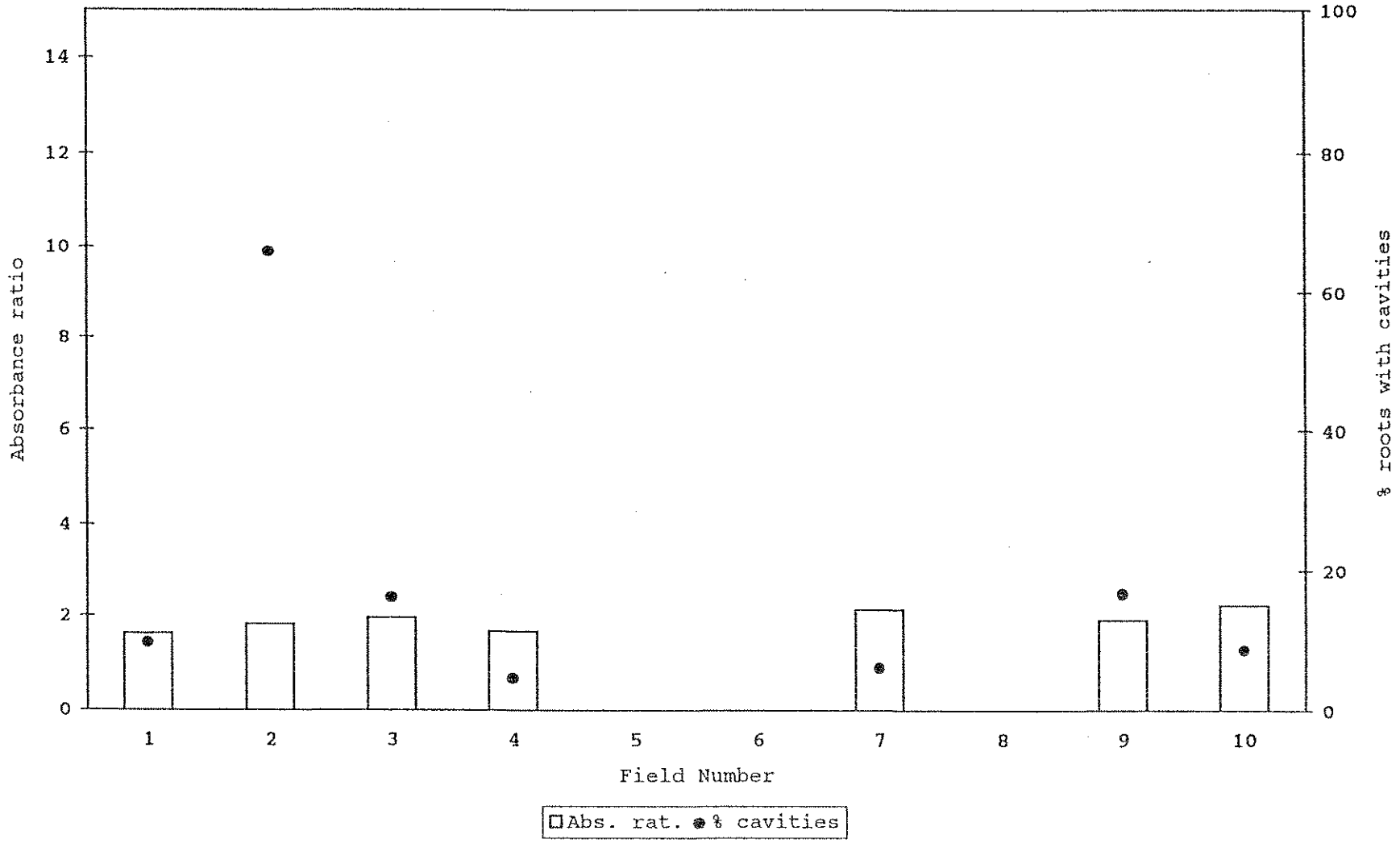


Fig.13 Absorbance ratios for *Pythium violae*, plus percentage cavity spot, for Knights fields in the 1992 survey based on soil and carrots collected in November

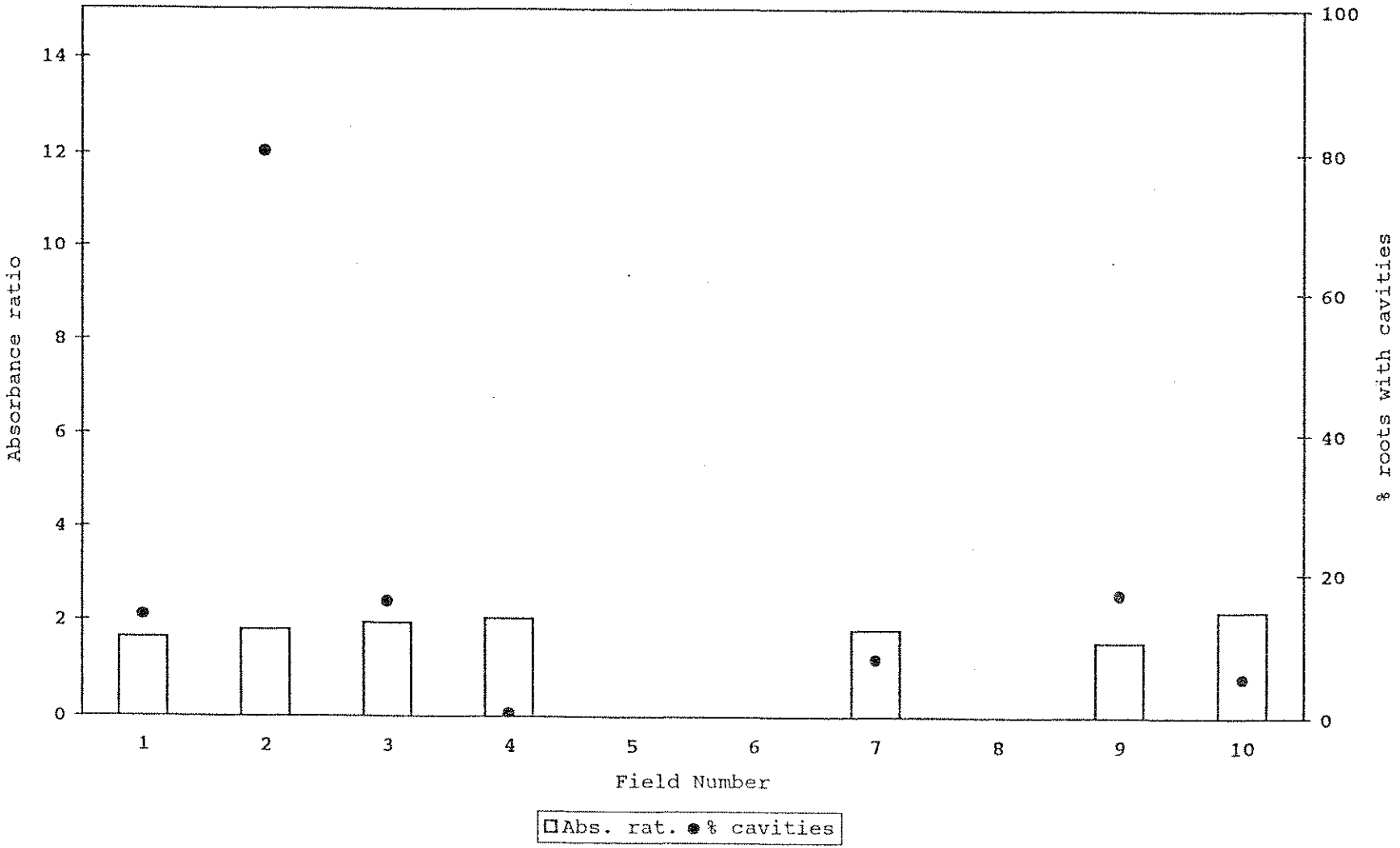
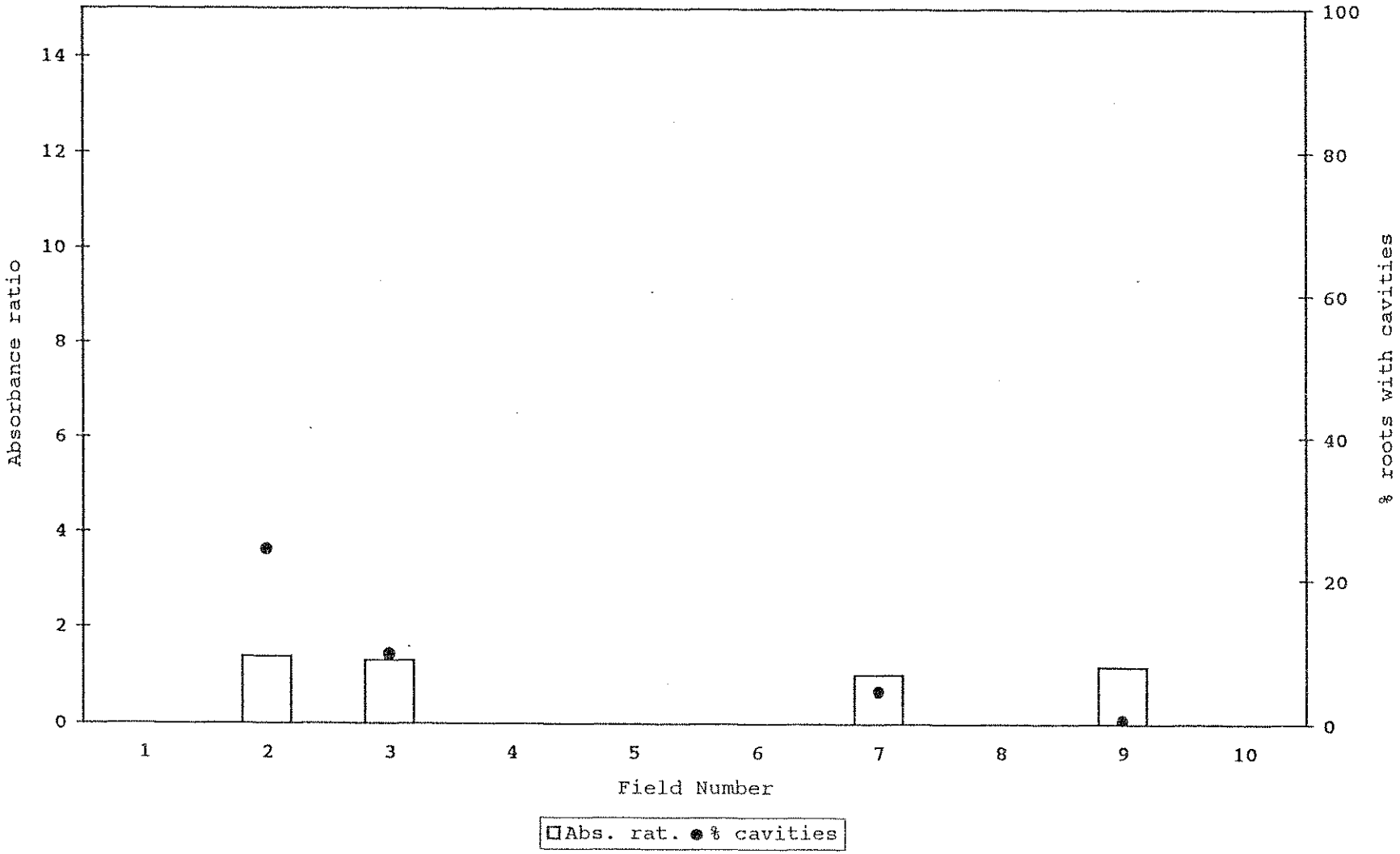


Fig.14 Absorbance ratios for *Pythium violae*, plus percentage cavity spot, for Knights fields in the 1992 survey based on soil and carrots collected in February



Cross reactivity of polyclonal antisera

Because polyclonal antisera were used throughout this study, cross reactivity between the antisera raised against the two species of *Pythium* must be considered. Preliminary tests had shown that there was little cross reactivity between the antisera raised against *P violae* and *P sulcatum* (Lyons & White, 1992). Over the whole of the present exercise, with minor exceptions, there was no correlation between the readings obtained with the two antisera. Absorbance values from the antisera raised against *P sulcatum* were generally higher than those for *P violae*, but disease caused by the former occurred only in one field. It is possible that the *P sulcatum* antisera recognise another fungus which is common in soil, but not of relevance to cavity spot. Alternatively, it could be that the threshold absorbance values prior to outbreaks of cavity spot caused by *P sulcatum* are much higher than those for *P violae*. Very little is known about either of the cavity spot pathogens, and within the present project it was not possible to fully explore this aspect.

Soil pH

Soil pH varied from 6.0 to 8.3 in the 176 plots surveyed in year 1 (Appendix Table 5). Only in 16.5 % of samples did the pH exceed 8.0, the value above which significant limitation of disease development by high ph could be expected. Overall there was a low correlation ($r = -0.103$) between soil pH and final percentage of cavity spot, indicating a complex and non-linear relationship.

For the 40 plots in the survey in year 2, soil pH varied from 4.8 to 7.7 (Appendix Table 6). The correlation coefficient was again low (r = 0.273) indicating a non-linear relationship, with most values for percentage cavity spot > 5 being associated with ph values in the range 6.5 to 7.8.

Pythium oligandrum

P oligandrum was assessed at each soil sampling, but only the pre-drilling data are presented (Appendix Table 5). In year 1 the number of cfu/g soil ranged from 0 to 271 over the 176 plots. The relationship between the levels of *P oligandrum* and cavity spot development is not simple. The correlation coefficient between those parameters was low (r = 0.099) indicating a non-linear relationship, or absence of any relationship. In field 1, where serious cavity spot caused by *P sulcatum* was found, *P oligandrum* was absent, probably because of repeated use of metalaxyl in the preceding years. Conversely, in field 8 where the most serious cavity spot caused by *P violae* was seen, the mycoparasite was present at the moderate level of 69.8 cfu/g soil. It is possible that a high population of *P violae* supported the growth of *P oligandrum*, but higher levels of *P oligandrum* were found in other fields where cavity spot was absent.

In the smaller survey in year 2, the levels of *P oligandrum* varied from 0 to 227 cfu/g soil (Appendix Table 6), but the fungus was absent from 50 % of the plots. The correlation with cavity spot was low (r = 0.106). *P oligandrum* was present only

at moderate levels in field 2 where the most serious cavity spot occurred. The highest levels of the mycoparasite were found in field 5 where insignificant levels of cavity spot were observed.

Cropping histories

There were no obvious links between previous crops and the percentage of carrots which developed cavity spot. In year 1, severe cavity spot developed in field 8 which had produced only two high organic matter crops (organic matter returned to soil is reputed to increase cavity spot) in the preceding five years. On field 1, where *P sulcatum* was causal, there had been four high organic matter crops in the same period. However, field 24 had also been cropped four times with high organic matter crops, but throughout the survey, including sampling in February after the crop had been covered with straw, disease was light.
In year 2, the highest percentage of carrots with cavities was in field 2 which had produced only one high organic matter crop in the preceding five years. Unfortunately only a quarter of the relevant data was available for the fields in this year, so firm conclusions cannot be drawn.

Pilot commercialisation of the diagnostic test

Following the commercially important results in 1991-92, HRI and Knights took part in a large scale pilot assessment of the diagnostic test, for which Knights provided the funding. Sampling and assays were as described above for year 2. Because absorbance

ratios would predictably be low during the dry summer months, it was felt important to start sampling only after there had been significant autumn rain. Late October was chosen on an *ad hoc* basis as the time when the first samples would be taken. The basis of the exercise was that Knights would sample prospective carrot fields and HRI would process those samples through the ELISA system. There would be no assessment of the results for most fields other than that done by quality control in the packhouse. The exercise was too large to undertake counting and grading of cavities using the HRI system.

A total of 62 fields representing 1989 acres were sampled and processed by ELISA. The sampling period was September to December and the results were all communicated by the third week in March 1993. For commercial reasons, sampling took place over a protracted period, and results were communicated over a period of 4 months.

Because of commercial considerations it is not possible to present the data in detail, however, the range of absorbance ratios was similar to those obtained in the above work. The assessment of disease in the two years was consistent in that low absorbance ratios were consistently associated with low disease at grading. High absorbance ratios were mostly associated with high disease levels in untreated strips in fields which were routinely treated with metalaxyl and mancozeb. Many fields which had consistently low absorbance ratios were cropped without recourse to applications of metalaxyl fungicide. Between the

Conclusions

1. Competition ELISA with polyclonal antisera raised to cell wall/membrane or cytoplasmic antigen of *P violae* produced a reliable indicator of the risk of cavity spot in field soil over three years. The process is commercially valuable in that most cavity spot outbreaks are caused by *P violae*.

2. Where absorbance ratios were consistently below 1.5, cavity spot was generally at low levels and it is doubtful if the use of fungicide would result in financial benefit.

3. Where absorbance ratios were consistently over 2.0, with some as high as 4.0 or 5.0, there was risk of the disease becoming established, and with the longer crop, potentially resulting in complete loss. These cases therefore required treatment with fungicide at the recommended rate, and, in accordance with label instructions. There was evidence that selection of cultivars with some field resistance and manipulation of drilling and harvest dates could help to reduce the risk of disease.

4. Absorbance ratios consistently higher than 4.0-5.0 up to the theoretical maximum of 17.5 indicate extremely high risk and the field should not be cropped with carrot in that season.

5. Polyclonal antisera raised against *P sulcatum* did not indicate risk in the one field where that fungus caused an outbreak of cavity spot. At the present time, we are unable to interpret results with antibodies raised to *P sulcatum*.

Glossary

Absorbance ratio - the ratio derived by dividing the absorbance values derived in ELISA for freeze-dried and non-concentrated soil extracts.

Antigen - material such as cytoplasmic extract of fungus which when injected into a mouse or rabbit results in the production of antibodies in that animal.

Antibody - see below.

Cavity spot - the major disease of carrot in temperate countries which is caused by slow-growing *Pythium*. The disease is initially seen as pale sunken lesions which over a short period discolour and are enlarged by secondary pathogens, bacteria and nematodes.

ELISA - enzyme-linked immunosorbent assay is a process based on the use of antibodies by which biological material may be quantified, with results being measured as differential colour intensity by automatic readers. The process facilitates the rapid screening of large numbers of samples.

Competition ELISA - a particularly sensitive version of the process used throughout the present work.

Genstat - the computing language used to process all data in this project.

Monoclonal antibody - an antibody produced from a single cell recovered from an immunised animal. MAbs were reputed to be of high specificity and therefore to be of particular value in the type of work central to this project.

Polyclonal antisera - whole antiserum taken from an immunised animal.

For explanation of any technical aspects of the report please contact the authors in writing.

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Appendix

Appendix Table 1

Field numbers and names, soil associations and cultivars sown for fields in 1991-92 survey

Field number and name		Soil association		Cultivar
1	Barn Battles	343f	Newmarket 1	Primo
2	Sanderson	343f	Newmarket 1	Nandor
3	Carters small wet	343f	Newmarket 1	Nandor
4	Hockwold No. 7	861b	Isleham 2	Nandor
5	Carter wetland	343f	Newmarket 1	Nandor
6	Langer long	343f	Newmarket 1	Predor
7	Battles	343f	Newmarket 1	Nairobi
8	Castellotti	343f	Newmarket 1	Nandor
9	Sanderson No. 2	343f	Newmarket 1	Nandor
10	Rix	343f	Newmarket 1	Nandor
11	Threshpits	343f	Newmarket 1	Nandor
14	Thompson Q	554b	Worlington	Fancy
15	Thompson P	554b	Worlington	Fancy
16	Thompson W	554b	Worlington	Nairobi
17	Thompson O	554b	Worlington	Fancy
18	Thompson X	554b	Worlington	Nairobi
19	Limekiln	343f	Newmarket 1	Nairobi
20	Foulden Breck	343f	Newmarket 1	Nairobi
21	Middle Shift	343f	Newmarket 1	Senior
22	Carters behind the barn	343f	Newmarket 1	Nandor
23	Barn Battles Big	343f	Newmarket 1	Nairobi
24	Big Long	343f	Newmarket 1	Nairobi
25	Small wooded	343f	Newmarket 1	Nandor
26	Cross Roads Drew	343f	Newmarket 1	Nandor
27	Narborough Front Bungalow	861b	Isleham 2	Nairobi
28	Narborough Middle Bungalow	861b	Isleham 2	Nairobi
30	West Acre Muck Heap	554b	Worlington	Nairobi
31	West Acre Stone Pit	554b	Worlington	Narbonne
32	Haywards 1	343g	Newmarket 2	Chantenay
33	Haywards 2	521	Methwold	Chantenay
34	Haywards 3	521	Methwold	Chantenay
35	Haywards 4	554b	Worlington	Chantenay
36	Haywards 5	554b	Worlington	Chantenay
37	Marham Red Barn	343f	Newmarket 1	Nairobi
38	Langer Steel Barn	343f	Newmarket 1	Predor
39	Carter Strawberry	343f	Newmarket 1	Nandor
40	Langer Cley Road	343f	Newmarket 1	Nandor
41	Allingham A	554b	Worlington	Nandor
42	Allingham B	554b	Worlington	Nandor
43	Allingham C	861b	Isleham 2	Nandor
44	Allingham D	861b	Isleham 2	Nairobi
45	Allingham E	861b	Isleham 2	Fancy
46	Stanhoe A	581f	Barrow	Fancy
47	Stanhoe B	581f	Barrow	Fancy

Appendix Table 2

Cropping history for fields in 1991-92 survey

Field No.	1986	1987	1988	1989	1990
1	Potato	Carrot	Beet	Barley	Potato
2	Barley	Barley	Beet	Potato	Wheat
3	Not available.....				
4	Beet	Barley	Parsnip	Grass	Grass
5	Not available.....				
6	Not available.....				
7	Potato	Carrot	Beet	Barley	Potato
8	Barley	Beet	SBarley	Wheat	Potato
9	Potato	Wheat	Beet	Peas	Wheat
10	Not available.....				
11	Barley	Carrot	Parsnip	Grass	Grass
14	Not available.....				
15	Not available.....				
16	Not available.....				
17	Not available.....				
18	Not available.....				
19	Turnip	Parsnip	Grass	Grass	Grass
20	Parsnip	Carrot	Swede	Grass	Barley
21	Parsnip	Carrot	Swede	Grass	Barley
22	Not available.....				
23	Lucerne	Lucerne	Beet	Potato	Barley
24	Carrot	Beet	Onion	Barley	Beet
	Carrot	Potato	Beet	Potato	Barley
25	Barley	Barley	Carrot	Beet	Barley
26	Grass	Barley	Carrot	Beet	Barley
27	Onion	Wheat	Carrot	Beet	Barley
28	Wheat	Onion	Carrot	Potato	Wheat
30	Barley	Barley	Beet	Barley	Barley
31	Barley	Barley	Beet	Barley	Barley
32	Not available.....				
33	Not available.....				
34	Not available.....				
35	Not available.....				
36	Not available.....				
37	Beet	Rye	Rye	Grass	Beet
38	Not available.....				
39	Not available.....				
40	Not available.....				
41	Beet	Potato	Barley	Beet	Onion
42	Barley	Beet	Carrot	Potato	Potato
43	Beet	Barley	Potato	Barley	Beet
44	Barley	Beet	Onion	Barley	Potato
45	Beet	Barley	Barley	Beet	Onion
46	Parsnip	Barley	Lucerne	Lucerne	Carrot
47	Carrot	Lucerne	Lucerne	Barley	Carrot

Appendix Table 3

Field numbers and names, soil associations and cultivars sown for fields in 1992-93 survey

Field no.	and name	Soil association		Cultivar
1	Janes Wood 32	343f	Newmarket 1	Nairobi
2	The Entry	554b	Worlington	Nairobi
3	Wilkins Heath	554b	Worlington	Nairobi
4	Heygate	343f	Newmarket 1	Nairobi
5	Parkers L-Shape	711f	Wickham 2	Narbonne
6	13 Acre	861b	Isleham 2	Nandor
7	Bone Mill	861b	Isleham 2	Nairobi
8	River Meadow	861b	Isleham 2	Nandor
9	Grange	861b	Isleham 2	Nairobi
10	Top Camp	343f	Newmarket 1	Nairobi

Appendix Table 4

Cropping history for fields in 1992-93 survey

Field no.	1987	1988	1989	1990	1991
1	Not available.....				
2	Swede/ Turnip	Carrot	Grass	Fallow	Lettuce
3	Not available.....				
4	Not available.....				
5	Not available	Oilseed Rape	Wheat	Beans	Linseed
6	Not available	Potato	Carrot	Sugar beet	Barley
7	Not available	Not available	Grass	Beet	Potato
8	Not available	Not available	Grass	Beet	Potato
9	Not available	Not available	Grass	Grass	Grass
10	Not available	Not available	Not available	Not available	Barley

Appendix Table 6

Soil pH and counts of *Pythium oligandrum* for fields in 1992-93 survey

Field	pH	*P. oligandrum* CFU/g
1	7.6	79.8
2	6.9	21.4
3	6.5	0.0
4	5.4	0.0
5	7.3	162.6
6	7.6	0.0
7	7.6	31.9
8	5.1	5.0
9	6.3	0.0
10	7.1	2.8

Appendix Table 5

Soil pH and counts of *Pythium oligandrum* for fields in 1991-1992 survey

Field	pH	*P. oligandrum* CFU/g
1	6.7	0.0
2	7.4	50.4
3	7.2	179.4
4	7.7	41.3
5	7.6	11.5
6	7.6	7.4
7	7.8	11.4
8	7.6	69.7
9	7.7	118.8
10	7.3	26.3
11	7.8	22.8
14	7.8	14.4
15	7.8	5.2
16	7.5	34.4
17	7.8	5.8
18	7.6	0.0
19	8.0	19.0
20	7.9	26.2
21	7.9	19.8
22	7.3	21.9
23	7.8	0.0
24	7.9	19.0
25	7.8	50.8
26	7.9	0.8
27	7.9	119.1
28	7.9	110.9
30	7.9	17.9
31	8.1	9.6
32	7.9	32.8
33	7.6	7.5
34	7.8	1.4
35	7.2	19.6
36	7.7	27.3
37	7.7	9.2
38	7.9	20.7
39	7.3	16.9
40	8.0	35.1
41	7.8	26.0
42	7.6	19.6
43	7.1	4.3
44	7.3	23.7
45	7.2	3.6
46	8.0	6.1
47	7.9	169.9

extremes, there was little tendency for the test to indicate false negatives, although for some fields where absorbance ratios were higher than average, disease levels were not always high (some false positives). This could be successfully handled commercially by manipulation of drilling and harvesting dates. Results from soil samples taken at the start of the sampling period, or up to four months later were seen to be similar in scale, with little or no effect on the prediction. It was concluded that the potential financial benefits of the system far outweighed the posibility of some unexpected disease on a small number of fields.

The first truly commercial samples were processed by Oxford Agricultural Consultants in spring 1994.