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ASPECTS OF SEED PHYSIOLOGY WHICH ARE INFLUENTIAL IN ESTABLISHMENT PROBLEMS OF VEGETABLE SEEDS

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The work presented in this thesis is
the result of my own investigations
and has neither been accepted nor is
being submitted for any other degree.

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...29th March 1972......Date

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GENERAL INTRODUCTION

GENERAL INTRODUCTION

Pre-emergence failures of pea seeds are most frequently reported in early spring, when the seeds encounter conditions of low temperature and high soil moisture in the field. These emergence failures occur even in seed lots that have germination capacities greater than the 80 percent declarable minimum. The absence of good correlations between laboratory germination tests and field emergence data, indicated that factors other than viability influenced the field emergence of pea seeds (Perry, 1970).

The advent of precision sowing, and the stringent timing requirements of the food processing industry has focussed attention on the need for a better understanding of the factors that influence the field emergence of pea seeds so as to enable more accurate predictions of field emergence to be made.

Seed peas are sold commercially as seed lots, a lot being a bulk of seed not exceeding 20 tons (Seeds Regulations, 1961) which has usually been taken from one crop. Two attributes of a seed lot influence its emergence in the field: the percentage of viable seeds in the seed lot and the vigour of the viable seeds (Heydecker, 1968). The first attribute can be and usually is determined by normal seed testing methods, but the vigour of seeds is more difficult to assess.

Various methods have been used to measure vigour in seed peas (Isely, 1957; Heydecker, 1965). Assessments of the performance of seeds under stress conditions in the laboratory have been tried with some success. Clark and

Baldauf (1958) found that the percentage of seeds emerging from field soil in the laboratory at a low temperature correlated well with field emergence. Caldwell (1960) found a similar correlation using emergence in laboratory soil at high temperature and high moisture. More recently, Perry (1968) evaluated the vigour of seedlings in a modified germination test in which the seeds were germinated in a standardized sterile medium, and seedling growth was measured after several days.

The relationship between seed exudation and infection by fungal pathogens, which is a common cause of emergence failures, has been reported on several occasions (Barton, 1957; Thomas, 1960; Flentje and Saksena, 1964). Matthews and Bradnock (1968) recognised that the relationship between exudation and emergence presented the possibility of a routine test for seed vigour, and they devised a laboratory test, based on the amounts of electrolytes seeds exuded into water. This test enabled the detection of seed lots of low planting value.

A third type of vigour test for seeds, based on seed respiration rates during the early stages of imbibition, was shown by Woodstock (1966) to give a reliable indication of the field performance of seed lots of maize.

The vigour tests so far mentioned can very usefully distinguish seed lots with a high potential field emergence from those with a low potential emergence (Heydecker, 1968). However, many seed lots do not lie clearly in either group since they show low emergence during adverse conditions but are capable of high field emergence under some sowing conditions (Perry, 1970).

This lack of complete agreement between vigour tests and the field emergence of seed lots emphasised the point made by Heydecker, (1968) that there are always two sides to the vigour problem, namely, the quality of the seed and the quality of the environment.

The work of this thesis is concerned with the influence of soil factors on the field emergence of pea seeds and the interaction of these factors with seed quality.

SECTION I

PHYSIOLOGICAL COMPARISONS OF SEED LOTS

INTRODUCTION.

Seed lots of some crop seeds, although exhibiting high viability in laboratory germination tests, produce a low number of seedlings in the field. These discrepancies between laboratory germination and field emergence have been attributed to variations in physiological vigour of seeds. Seed vigour, although a vague term, has become accepted by seed technologists through common usage, (Heydecker, 1965). Recently, attempts have been made to define vigour more accurately, and the definition most relevant to the present study is one proposed by Perry (1968), "Vigour is the property which controls the ability of seeds to germinate, and produce seedlings, under adverse field conditions."

The importance of vigour in the field situation has been demonstrated by Thomas (1960) for castor beans, Pollock & Toole (1966) for lima beans, and Perry (1968) for peas, all of whom found that under adverse conditions seeds of low vigour produced lower stands than seeds of high vigour.

Laboratory determinations of vigour are therefore useful in differentiating seeds of doubtful planting value, and it is recognized that laboratory tests, where seeds are germinated under stress conditions, are one way of obtaining estimations of seed vigour. The most important stress test was the cold test for maize (Hoppe, 1953), but although still important, its use has diminished largely because of difficulties involved in standardization, (Harper and Landragin, 1955).

Other types of vigour test have been based on physiological or other characteristics of seeds that can be easily measured in the laboratory, and that may be important during the germination of the seeds.

Woodstock & Feeley (1965) found a significant correlation between early respiration rate and subsequent seedling growth of maize grain, even when the respiration rates of the grains were measured after only 3-4 hours imbibition. Later, Woodstock (1965) produced a vigour test for maize grain, based on their initial respiration rates. Grain with high rates of oxygen uptake produced more seedlings in the field than grain with low oxygen uptake values.

The relationship between initial respiration rates of seeds, and seedling vigour, has been found to apply to other crop seeds besides maize. Injurious treatments to seeds of sorghum, radish and wheat (Woodstock & Justice, 1967), lima beans (Woodstock & Pollock, 1965), tomato, cabbage, snap bean, cotton, onion, and cacao have revealed that the resulting differences in vigour are usually reflected by differences in the respiration rates of the seeds, during the first six hours of imbibition.

Vigour tests, based on the amounts of material seeds exude into water, have also been shown to produce useful estimations of field emergence of different seed lots. Hottes and Huelson (1927) reported that maize grains of low vigour, and low field emergence, exuded large amounts of material when they were steeped in water. This finding was confirmed by Tatum (1954) who suggested that the material exuded into the soil may promote the growth of fungi. Significant negative correlations between electrical conductivity of steep water, and emergence in soil, were found for castor beans (Thomas, 1960) and peas (Matthews and Whitbread, 1968) and later an exudation test was suggested (Bradnock & Matthews, 1970), whereby an indication of the field emergence of a seed lot of peas could be obtained by measurements of the exudation of electrolytes from the seeds. They demonstrated that the test provided a more reliable estimation of field emergence than did the standard germination test.

Before embarking on work to examine the response of seed lots, differing in field emergence potential, to different field conditions, an attempt was made to examine the vigour of the seed lots by means of both respiration and exudation tests. The aim of the work in this section was therefore twofold. Firstly, to assess the value of the existing vigour tests in estimating the potential field emergence of eight seed lots of peas, cultivar, Dark Skinned Perfection, and secondly, to classify the eight seed lots, in terms of relative vigour, for future laboratory and field experiments.

MATERIALS.

Eight commercial seed lots of Dark Skinned Perfection, a wrinkle-seeded pea, were used. Details of their country of origin, year of harvesting and supplier are given in Table 1.

Table 1. Origin, year of harvesting and supplier of the eight seed lots used in the investigation.

Seed lot number	Country of Origin	Year of Harvesting.	Supplier.
39	New Zealand	1967	Charles Sharpe & Co. Ltd.
47	New Zealand	1967	"
04	New Zealand	1967	"
03	New Zealand	1966	"
54	Not known	Not known	Unilever Research Lab.
80	" "	" "	"
21	" "	" "	"
20	" "	" "	"

METHODS

(1) Laboratory Germination.

Four replicates of each seed lot, each containing 50 seeds, were placed in moistened, rolled paper towels and incubated at 20°C. Seedlings without a main shoot, or without a primary root and less than two secondary roots, were excluded from the percentage germination, together with seeds that developed abnormally because they were broken or decayed.

(2) Field Emergence.

Two hundred seeds from each seed lot were sown on April 24th, 1970, the mean maximum air temperature for the week following being 50°F, and the total rainfall being 0.2 mm. This planting was one of the nine plantings which made up the experiment described in Section II. The seeds were sown in four randomised blocks, each containing 50 seeds from each seed lot. The eight rows of seed in each block were 12 feet long, and seeds were planted at a depth of 1½ in. Emergence counts were made until no further emergence occurred, in this case after 45 days.

(3) Rates of Respiration.

The respiration rates of the seeds were measured as the rates of oxygen uptake and carbon dioxide evolution in microlitres per hour at 20°C, using a Gilson Differential Respirometer. Samples of four seeds were placed in 17 ml. reaction vessels with 2 ml. of distilled water. One empty flask, a thermoblank, was included in each test, following the recommendations of Carver & Gloyne (1971).

The respiration rates of the seeds were measured after

24 and 43 hours imbibition, the seeds having previously been incubated at 20°C for the required period, on filter papers in Petri dishes containing 16 ml. of water, with ten seeds per dish. The formula used to convert the relative volume changes measured on the instrument, to microlitres of gas at standard conditions, is given in Appendix I.

(4) Exudation of Electrolytes.

Four replicates of 20 seeds from each seed lot were soaked in 200 ml. of deionized water at 15°C. The conductivity of the seed steep water was measured after 24 hours, (Matthews & Whitbread, 1967) using a Portland Conductivity meter.

Individual exudation tests were carried out on 300 individual seeds from each seed lot, the conductivity being measured after the seeds had been soaked in 10 ml. of deionized water in 3 in. by 1 in. tubes at 15°C for 24 hours.

(5) Enzyme/Reducing Sugar Test.

This test was a modification (Matthews & Carver, 1971) of the viability test for rape, proposed by Takayanagi & Murakami (1968). Exudates were produced from each of the seed lots by soaking 20 seeds in 100 ml. of distilled water at 15°C. After 24 hours, one ml. of soak water was mixed with 0.1 ml. of invertase concentrate to convert the sucrose present in the pea exudate, to glucose and fructose. A Clinitest reaction tablet was added to each tube and the mixture was shaken, 15 seconds after the effervescence had stopped. The colours of the final solutions were compared with standard colours, and an assessment of the concentrations of glucose present in the solutions, was made.

RESULTS

(1) Laboratory Germination and Field Emergence.

The seed lots all exhibited high levels of laboratory germination, higher than the declarable minimum of 80%, the mean percentage germination, as can be seen from Table 2, ranged from 85-96%.

Besides field emergence, the percentage mortality of the seed lots in the field is also presented in Table 2 and was calculated as follows:-

$$\frac{\text{\% Lab. Germination (viability) - \% Field Emergence}}{\text{\% Lab. Germination.}} \times 100$$

The field emergences of seed lots 80, 21 and 20, corrected for the differing viabilities of the lots, that is, calculated as the percentage of viable seeds that emerged, were very low, less than 20% and seed lot 20 exhibited 97% mortality. In contrast, seed lot 39 showed no field mortality.

On the basis of field emergence, the following classification of the seed lots was made. Seed lots 39 and 47, with very high field emergences, greater than 90% were termed good, lots 04, 03 and 54, with field emergences between 60% and 90%, were termed intermediate, and lots 80, 21 and 20, poor, as they showed field emergences of less than 20% of the seeds sown.

(2) Respiration Rates.

The oxygen uptake and carbon dioxide evolution data are presented in Table 2 as microlitres of gas per g. dry weight of seed per hour. The oxygen uptake rates of the seed

Table 2. Comparisons of performance of eight seed lots of pea, cultivar Dark Skinned Perfection.

Seed Lot No.	CO_2 evolution ul/h.g.		O_2 Uptake ul/h.g.		R.Q.		Exudation in µmho.		% Laboratory Germination	% of viable seed emerging in the field	% mortality in field.
	24 hrs.	43 hrs.	24 hrs.	43 hrs.	24 hrs.	43 hrs.	300 single (Mean)	Bulk Test			
39	249	233	174	307	1.52	0.71	58	158	93	100	-
47	176	276	163	255	1.66	1.09	76	207	95	92	7
04	263	321	147	329	1.69	1.07	72	278	85	62	38
03	191	256	176	286	1.10	0.89	50	197	96	86	13
54	252	265	152	307	1.65	0.99	40	190	96	75	25
80	420	263	95	188	5.22	1.47	176	490	90	18	82
21	198	210	96	185	3.08	1.17	125	480	90	7	93
20	239	358	139	134	1.43	3.69	155	475	85	2	97

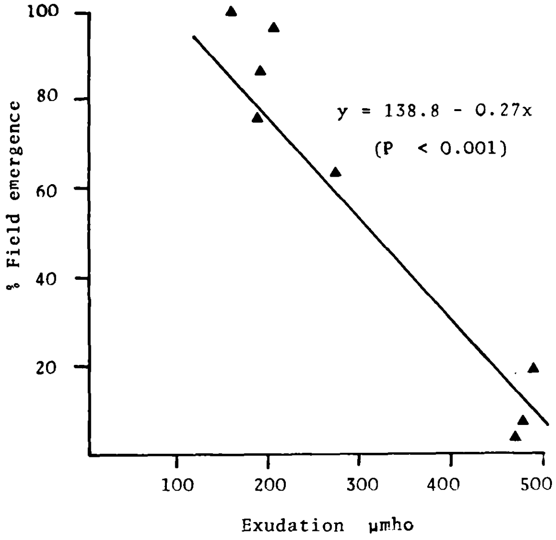
lots, after 24 hours imbibition ranged from 95-176 μl/h.g. dry weight, and seed lots with a high field emergence had distinctly higher levels of uptake than seed lots exhibiting low field emergence. The differences between the good and intermediate seed lots were less marked, however, and lot 03, the intermediate lot with the highest field emergence, had a higher uptake than the two good seed lots 39 and 47. Nevertheless, the relationship between oxygen uptake and field emergence (r = 0.819*) presented in Table 3 and Figure 1 was significant. This relationship appeared closer (r = 0.842**) when the oxygen uptake after 43 hours was used, but again the oxygen uptakes of the intermediate and good seed lots were similar. In Figure 1, the points fall into two groups indicating the similarity between the good and intermediate seed lots, as opposed to the poor lots.

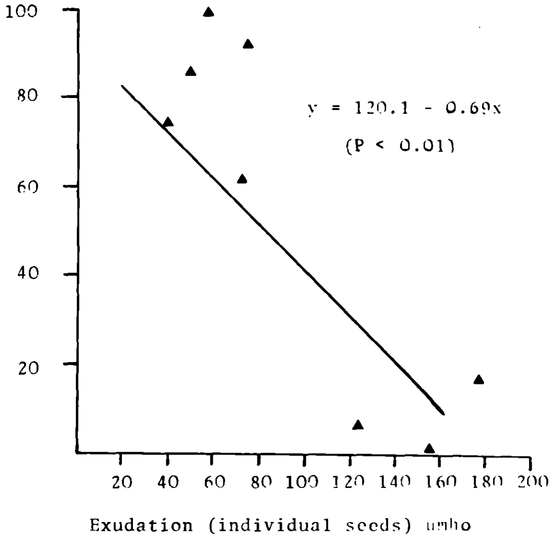
Table 3. Correlation Coefficients relating parameters measured in the laboratory, to field emergence of the eight pea seed lots, cultivar, Dark Skinned Perfection.

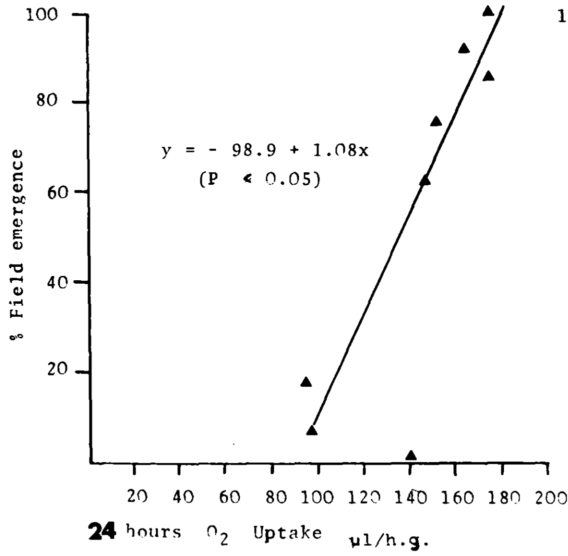
Lab. Assessments Correlated with Field Emergence	Correlation Coefficient.
Laboratory Germination	0.629 N.S.
Bulk Exudation	-0.959 ***
Individual Exudation	-0.842 **
Carbon dioxide output (24 hrs.)	0.306 N.S.
Carbon dioxide output (43 hrs.)	0.298 N.S.
Oxygen uptake (24 hrs.)	0.819 *
Oxygen uptake (43 hrs.)	0.842 **
Respiratory Quotient (24 hrs.)	0.497 N.S.
Respiratory Quotient (43 hrs.)	0.700 N.S.

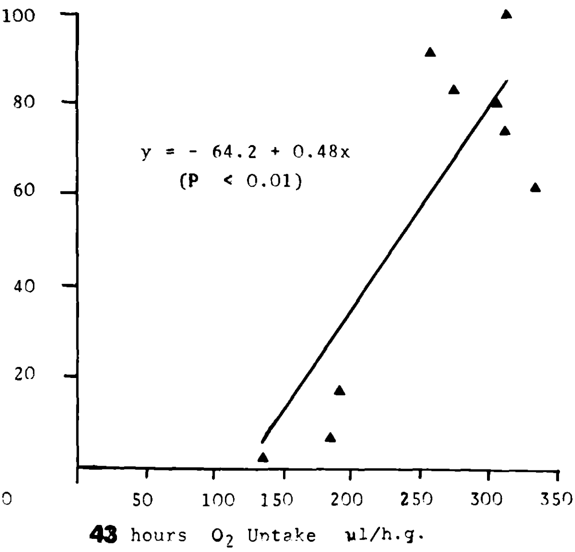
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

Fig. 1. Linear regressions of percentage field emergence of the 1970 seed lots on the electrical conductivity of seed steep water of bulk tests and individual seeds, and on the oxygen uptakes (µl/h.g.) of the seeds after 24 and 43 hours.


100
80
60
40
20
% Field emergence
y = 138.8 - 0.27x
(P < 0.001)
100
200
300
400
500
Exudation µmho


100
80
60
40
20
y = 120.1 - 0.69x
(P < 0.01)
20
40
60
80
100
120
140
160
180
200
Exudation (individual seeds) µmho


100
80
60
40
20
% Field emergence
y = - 98.9 + 1.08x
(P < 0.05)
20
40
60
80
100
120
140
160
180
200
24 hours O2 Uptake µl/h.g.


100
80
60
40
20
y = - 64.2 + 0.48x
(P < 0.01)
50
100
150
200
250
300
350
43 hours O2 Uptake µl/h.g.

The relationships of carbon dioxide evolution after 24 hours (r = 0.306) and 43 hours (r = 0.298) to the emergence of the seed lots in the field, were not significant. The respiratory quotient (R.Q.) values obtained for the different seed lots after 24 and 43 hours imbibition did distinguish between good and poor lots, good seed lots having lower R.Q. values than poor lots, but as with the oxygen uptake values, the differences in field emergence between the good and intermediate lots were not reflected in differences in R.Q.

(3) Exudation Tests.

The bulk exudation test produced a highly significant negative correlation (r = -0.959***) between the exudation of electrolytes, and field emergence of the eight seed lots. A reliable estimation of the field emergence of the seed lots could be obtained therefore, by measuring the exudation levels of the seeds in the laboratory. Some difficulty again arose, however, in separating the good from the intermediate seed lots, on the basis of the electrical conductivity results, but there was no difficulty in distinguishing the three poor lots 80, 21 and 20 from the others, as they had levels of exudation two or three times higher than those of the good and intermediate lots.

The measurement of the exudation of electrolytes from individual seeds of the seed lots, produced a slightly less significant relationship (r = -0.842**) between exudation and field emergence, than the bulk exudation test produced. The seed from poor lots had higher levels of exudation than those from good lots, but once again, even though the results were the mean figures obtained from replicates containing 300 seeds, the good and intermediate lots did not differ markedly.

The results of the enzyme/reducing sugar test, shown in Table 4, showed that the amounts of glucose detected in the modified seed soak water varied from nil to 1 mg/ml and that the amount detected was an indication of the field emergence of the seed lot.

Table 4. Comparison of laboratory germination, field emergence, and 'Clinitest' reaction for eight seed lots of pea, cultivar Dark Skinned Perfection.

Seed Lot	% Laboratory Germination	% Field Emergence	Colour of 'Clinitest' Sol.	Conc. of Glucose in mg/ml.
39	93	100	Blue	-
47	95	92	Blue	-
04	85	62	Blue/Green	< 0.5
03	96	86	Blue	-
54	96	75	Blue/Green	< 0.5
80	90	18	Yellow	1
21	90	7	Yellow	1
20	85	2	Yellow	1

This particular test was the only one that was able to distinguish some of the intermediate lots from the good lots. The intermediate seed lot that was not distinguishable from the good lots, lot 03, was the intermediate lot with the highest field emergence.

Assessments of the field emergence of the seed lots could be made by using any one of the three exudation tests, or by measuring the oxygen uptakes of the seed lots in the laboratory. However, only the enzyme/reducing sugar test was capable of distinguishing between good, intermediate and poor seed lots, the other tests with the seed lots used here, only allowed a distinction between good and poor seed lots to be made.

DISCUSSION

The discrepancies that can occur between the germination capacity and field establishment of pea seeds have been demonstrated. Seed lot 20, although showing a laboratory germination of 85% had a field emergence of 2%, whereas seed lot 39, with a laboratory germination of 93%, showed a 100% field germination of viable seeds sown. However, laboratory tests exist to detect seed lots with very low emergence potential.

The only instance of a respiration test for seed vigour being conducted on pea seeds, is that of Woodstock, referred to by Heydecker (1968). The results of the present study agree with the quoted findings of Woodstock in that seeds of high vigour, that had proven high field emergence, had higher rates of oxygen uptake than seeds of low vigour. Woodstock found a significant relationship between seed respiration and field emergence for seeds that had imbibed for as little as six hours. In contrast, preliminary experiments conducted on the eight seed lots used in this work, showed the uptake of the seed lots after six hours imbibition to be too variable to give a significant relationship. It was only after 24 hours and more particularly 43 hours imbibition that the relationship proved to be highly significant. It was also reported that Woodstock found the R.Q. values of the seed lots gave a better indication of field performance than the germination test, but in the present study, this was not the case. The respiration studies did indicate, therefore, that a respiration test for pea seed vigour, based on the oxygen uptake of the seed lots was possible. However, this type of test may not be capable of distinguishing between seed lots of high and intermediate vigour.

The bulk exudation test (Matthews & Bradnock, 1967) also gave a good indication of the potential field emergence of the seed lots. Heydecker (1968) felt, however, that vigour tests should be conducted on individual seeds from a seed lot, rather than bulk samples, as only in these circumstances would seed lots containing many good seeds and a few dead seeds, be distinguished from seed lots consisting of intermediate seeds. Hayman (1969) also suggested individual rather than bulk tests would be more meaningful, particularly in the case of exudation tests. However, the individual exudation tests conducted in this work, although being more time consuming, but using larger samples than the bulk tests, gave a slightly less significant relationship between exudation and field emergence, than did the bulk exudation test.

One criticism of the exudation test is that the amounts of electrolytes leached from seeds may not be the most 'relevant' measure relating exudation to field emergence. It has been suggested (Heydecker, 1968) that as sugars are known to encourage microbial colonization, a test for leakage of sugars from seeds may be more meaningful. However, the positive correlation found between electrical conductivity and amount of soluble carbohydrate in pea seed exudates (Matthews & Carver, 1971) suggested that the standard exudation test was indirectly measuring the release of sugars from the seeds, and that the alternative method, suggested by Heydecker, was not necessary. Nevertheless, the enzyme/reducing sugar test, which measured the release of sugars by seeds directly, did prove to give a better indication of field emergence than the other exudation tests. It was the only test conducted that was capable of distinguishing intermediate from good seed lots.

The work has therefore confirmed that the bulk exudation test, a modified exudation test developed during the course of the work and a respiration test based on the oxygen uptake of seeds gave valuable estimations of the potential field emergences of the eight seed lots.

It was also possible to classify the eight seed lots in terms of their relative vigour, or physiological condition, based on the results of these tests. Seed lots 39 and 47 were classified as good, lots 04, 03 and 54 as intermediate, and lots 80, 21 and 20 as poor. Although in all of the vigour tests, seed lot 03 was indistinguishable from the good seed lots, its field emergence did merit it being placed in the intermediate category.

The work of this section, clarified the differences in vigour of the seed material to be used in further studies of the influence of soil conditions on the emergence and physiology of pea seeds.

SECTION II

SOIL CONDITIONS AND SEED EMERGENCE

INTRODUCTION

Much of the previous work on the influence of soil conditions on the emergence of crop seeds has been on large-seeded crops, possibly because the size of the seeds made emergence failures more obvious. Dickson (1923) found that the attack of maize seedlings by Giberella zeae varied according to soil moisture and temperature. The fungus became more pathogenic at temperatures that were too low for the germination of maize grains. He also found that if soil moisture limited the germination of maize grains, then the fungus became more pathogenic over a wider range of temperatures.

Harper (1955), also working with maize, found that seed mortality was highest in conditions that just prevented or restricted seed germination, and he suggested that environmental conditions that were adverse to the host, but allowed the pathogen to remain active, caused the highest levels of pre-emergence mortality.

Previous work on pea pre-emergence mortality has concentrated on the influence of soil moisture on the incidence of failures. Hull (1937) noticed that rain shortly after seeds were sown caused high levels of seed mortality, and Jones (1931) and Angell (1952) also found that the addition of water to the seedbed after sowing, increased the levels of pre-emergence mortality.

Wellington (1962) investigated the discrepancies that occurred between the germination capacity and the field establishment of peas, and agreed with Harper's findings for maize, that the largest discrepancies were found when the period of germination was prolonged by low temperatures. Wellington also suggested that pre-emergence mortality at low temperatures was unlikely to be due to a direct effect of temperature since Torfasen and Nonneche (1959) found that pea seeds could tolerate prolonged exposure to 40°F in the absence of pathogens. He concluded that high soil moistures and low temperatures acted indirectly in producing the higher levels of pre-emergence mortality. A similar suggestion was put forward by Leach (1947), who suggested that increased levels of pre-emergence mortality occurred if the environmental conditions reduced the growth of the host relatively more than that of the pathogen, giving the pathogen a competitive advantage. In contrast, Hayman (1969) working on pre-emergence mortality of cotton, found that low temperatures reduced the growth rates of the pathogen and the host equally, and noted that low temperatures caused the host to exude more sugars and amino acids. These exudates, he suggested, favoured the growth of the pathogen and led to increased pre-emergence mortality.

Special attention has been paid to differences that occurred in pre-emergence mortality between different cultivars, subjected to the same environmental conditions. Harper (1955) found that much of the varietal resistance of maize to pre-emergence mortality was due to their differing abilities to germinate rapidly and grow at low temperatures. Woyke (1961) reported that low temperatures decreased the germination of wrinkle-seeded peas more than that of round-seeded peas, and Thomas (1960), working with castor beans, found that cultivars differed in the temperature that caused the maximum stand loss due to pre-emergence mortality. Perry (1967) and Matthews and Bradnock (1968), found that not only were there differences in pre-emergence mortality between different cultivars subjected to the same conditions, but also considerable differences existed within the same cultivar. Seed lots of the same cultivar showed marked differences in predisposition to pre-emergence mortality. Furthermore, they noted that high levels of pre-emergence mortality were associated with adverse soil conditions and that seed lots showing high predisposition readily exuded materials. Similar observations were made between different cultivars of peas (Flentje and Saksena, 1964) and of beans (Schroth and Cook, 1964). It is apparent, therefore, that in addition to soil moisture and temperature, seed condition is important in determining the incidence of pre-emergence mortality.

The aim of the work of this section was to investigate more fully the relationship between soil moisture and temperature, and the incidence of pre-emergence mortality in pea seeds. The relationship was investigated in the field, on three differing soil types, and in laboratory experiments where soil moistures and temperatures could be more carefully controlled. The interactions of seed condition and soil conditions in influencing the incidence of pre-emergence mortality was examined by using several seed lots differing in their physiological vigour (Section I) from within the same cultivar.

MATERIALS AND METHODS.

(1) Emergence in the Field.

Eight seed lots of the cultivar Dark Skinned Perfection were used,details of which are shown in Table 1.

The three areas designated for planting within the grounds of the University of Stirling, were chosen for their differing soil types. The Nursery and the Field were both loams, the latter with a higher organic content, and the Sword site was a silty loam that had previously been down to permanent grass. The moisture holding capacity against gravity of the three soils after sifting through a ¼ in. sieve were 33%, 34%, and 18% for the Nursery, Field and Sword respectively. The land was ploughed in autumn, and each site was cultivated with a rotary cultivator in spring.

The experiments were conducted from early Spring to Summer, 1970. The peas were sown by hand in 12 foot (3.7 m) rows, fifty seeds per row, at a depth of 1½ in. (3.8 cms). A replicate block measuring 12 feet by 10 feet (3.7 m by 3.1 m) consisted of one row of each of the seed lots, 1 foot (30.5 cms) apart, and at each planting date four replicate blocks were sown at each site. The seed lots were arranged randomly within the replicate blocks and the blocks were randomly sown within the designated sowing areas. Nine sowings were made on the following dates:- 27th March, 6th April, 24th April, 4th May, 13th May, 22nd May, 2nd June, 18th June and 1st July, at the Nursery and Field sites, but the last proposed planting was omitted from the Sword site, as on the day the soil was too dry.

Emergence counts were taken every few days until no further emergence occurred, which in the earlier sowings took forty-nine days, but in the June sowings took twenty-one days.

During the course of the experiments, a number of records were obtained from the meteorological station situated at the Nursery site. The maximum and minimum air temperature, 2 in. (5.1 cm) and 4 in. (10.2 cm) soil temperature were measured daily. The one foot soil temperature, and rainfall were also recorded daily. A mercury in steel thermometer recorded the soil temperatures at 2 and 4 in. continuously, and enabled the calculation of hours below selected temperatures, during the growing season.

(2) Emergence in Unsterilized Soil in the laboratory.

Soil was taken from the experimental garden area of the University of Stirling, an area referred to previously as the Nursery. The soil was air dried on benches in a heated greenhouse, sifted through a ¼ in mesh sieve and then stored in metal bins until required. The moisture content of the soil was 6%, the weight of water expressed as a percentage of the weight of the oven dry soil.

The five moisture contents required, 15%, 20%, 25%, 30% and 40%, were made up by adding measured amounts of water to the air dried soil. The soils of the required moisture content were mixed in bulk in large polythene bags and left to equilibrate for one week, in controlled temperature rooms at the four temperatures which the experiments were to be conducted, namely 5°C, 10°C, 15°C and 20°C. After one week the soil was placed in the seed trays and the seeds sown.

Five seed lots were used, one good lot 47, three intermediate lots, 04, 03, 54, and one poor seed lot 20, the classification being based on work reported in Section I. The complete experiment consisted of 200 trays, fifty at each temperature. Within these fifty trays were ten trays at each of the five moisture contents. Each tray held ten rows of seed, each row consisting of five seeds, so each seed lot was represented by two rows of seed, sown at random within the tray. The total number of seeds of each seed lot subjected to a combination of moisture content and temperature was one hundred.

The seeds were planted to a depth of 1 in. (2.54 cms) in the soil, and after planting the trays were covered with moisture-proof wrap to prevent moisture loss. The trays were then stacked in ten replicate blocks, in controlled temperature rooms. Only two of the temperature treatments could be conducted at any one time, due to lack of controlled temperature room space. The 5°C, and 10°C, experiments were completed before the 15°C and 20°C experiments.

The emergence of the seed lots was recorded and a number of seeds were removed and fungal isolations were made from them. Fungal isolations were done by washing soil from seeds with tap water, removing the testa and washing the cotyledons and embryo axis in three changes of sterilised distilled water. Each cotyledon was cut into four pieces and the axis and cotyledon pieces from each seed were then placed separately on plates of tap water agar and incubated at 20°C.

RESULTS

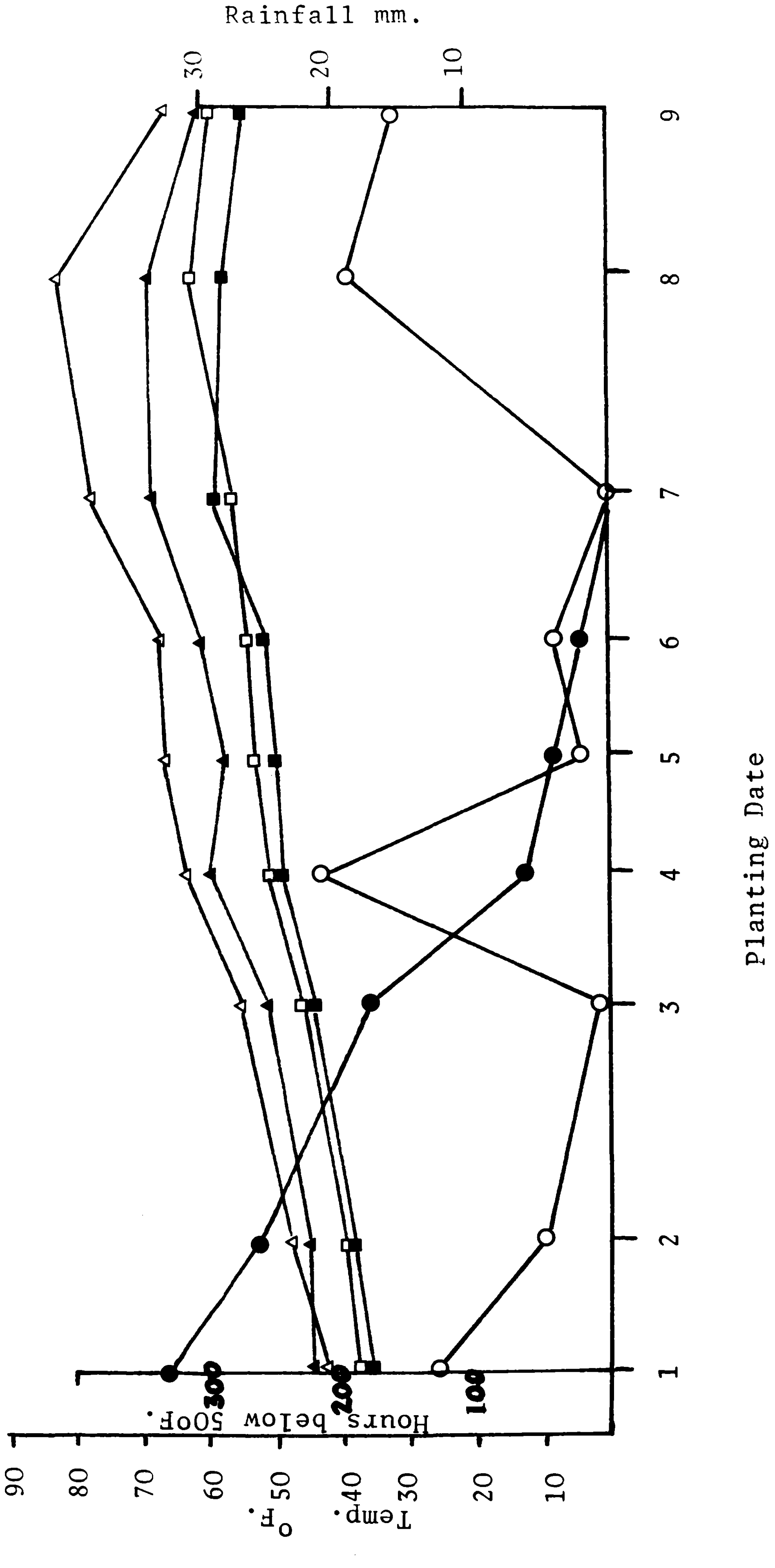
(1) The Influence of Planting date on Field Emergence.

The close proximity of the three experimental sites enabled the meteorological data recorded at the Nursery to be used for all the sites. Some of the measurements of soil temperature and rainfall that were obtained for the nine planting dates, are shown in Fig. 2.

The soil and air temperatures, presented as a 7 day mean temperature, starting on the day preceding planting, increased gradually through the season. The largest increases in temperature were recorded by the 2 in. (5.1 cm) soil thermometers, with the 4 in. (10.2 cm) and one foot (30.5 cm) readings increasing more slowly. Rainfall, measured as the total 7 days after planting, showed no overall pattern, but was greatest at planting dates one, four and eight. From the continuous recording thermometer, the total number of hours

Fig. 2. Values for the weather variables measured during the 1970 season. Variables are shown as follows:

- △ 2 in (5 cm) soil temperature (mean max. 1 day before and 5 days after planting).
- ▲ air temperature (mean max. 1 day before and 5 days after planting).
- □ 1 foot temperature (mean max. three days either side of planting.
- ■ 4 in (10 cm) temperature (mean minimum, 2 weeks after planting).
- ○ Rainfall (mm) (1 week after planting).
- ● Hours below 50°F (Total, two weeks after planting).


Rainfall mm.
30
20
10
Temp. °F.
90
80
70
60
50
40
30
20
10
Hours below 50°F.
300
200
100
Planting Date
1
2
3
4
5
6
7
8
9

below 50°F was calculated for each two week period following a planting, and this total decreased through the season until at planting dates seven and after, no temperatures below 50°F were recorded.

The emergence of the seed lots in the field and laboratory experiments was corrected for the viability of the lots, percentage emergence being expressed as a percentage of viable seed and the results were subjected to factorial analysis following angular transformation.

The ultimate emergence of the eight seed lots at the Nursery, Field and Sword sites are represented in Figures 3, 4, and 5 respectively. The similarity between the response curves of the seed lots at the three sites, shown more clearly in Fig. 6, was confirmed by the fact that the analysis of variance of the emergence results indicated that no significant differences between the number of seeds emerging at the different sites occurred. For this reason, the emergence at the field site will be discussed fully, and only deviations from this pattern will be mentioned for the Nursery and Sword sites.

The most striking feature of the ultimate emergence results was the manner in which the emergence of seed lots 04, 03, and 54 gradually increased through the season, similar to the increases in soil temperature shown in Fig. 2. Large increases in emergence were shown by these three seed lots until planting date four, after which they all maintained high levels of emergence, from 80 to 95%.

Seed lots 39 and 47 were relatively unaffected by planting date, being able to maintain high levels of emergence, even during the low soil temperatures of the earlier planting dates.

Fig. 3. The percentage field emergences of the eight seed lots planted at the Nursery site on nine occasions during the 1970 season. Seed lots are shown as follows:

39	●———————
47	○———————
04	□———————
03	■———————
54	△———————
80	▲———————
21	△—— —— ——
20	▲—— —— ——

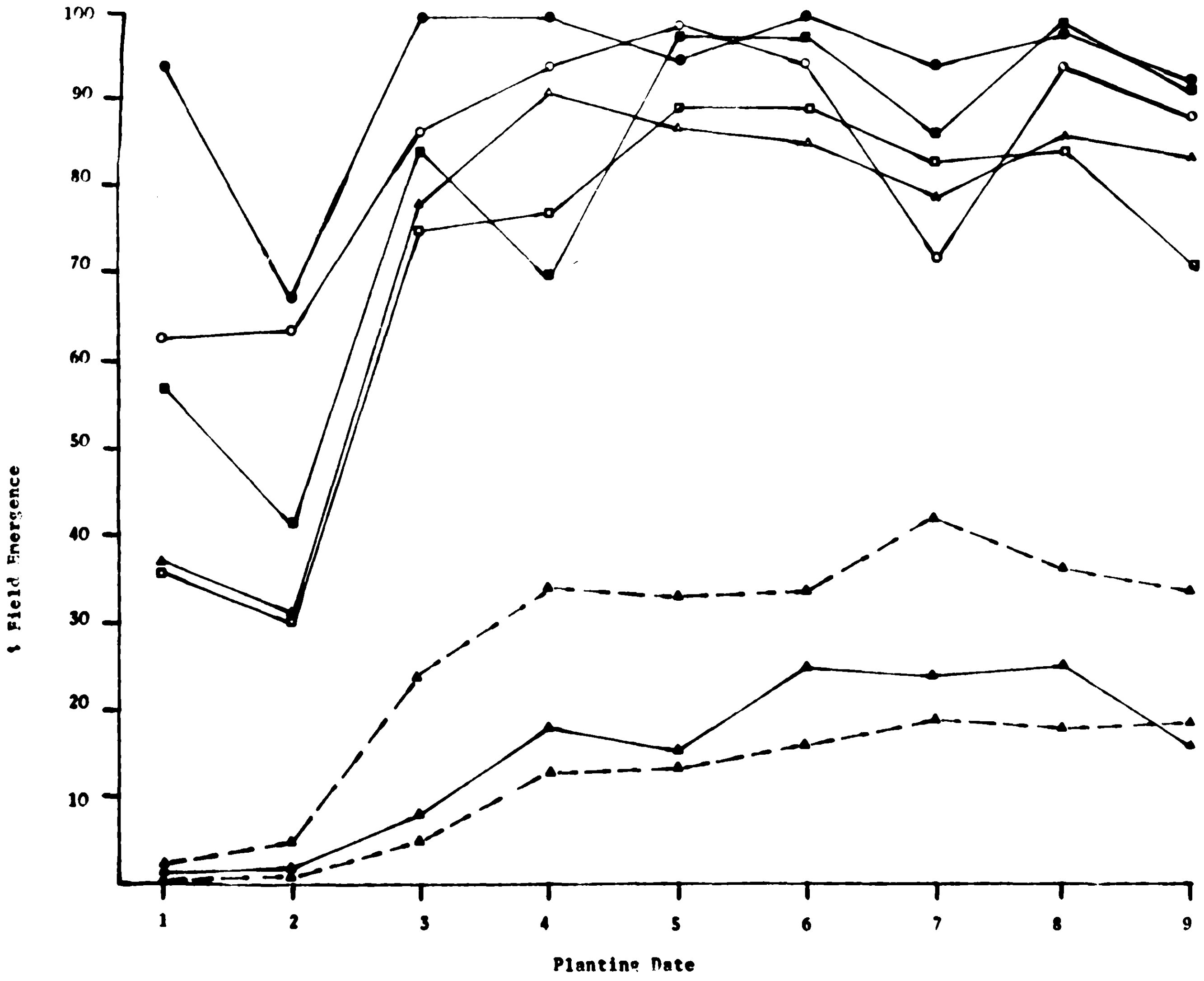

100
90
80
70
60
50
40
30
20
10
% Field Emergence
1
2
3
4
5
6
7
8
9
Planting Date

Fig. 4. The percentage field emergence of the eight seed lots planted at the Field site on nine occasions during the 1970 season. Seed lots are shown as follows:

39	●————————
47	○————————
04	□————————
03	■————————
54	△————————
80	▲————————
21	△——— ——— ———
20	▲——— ——— ———

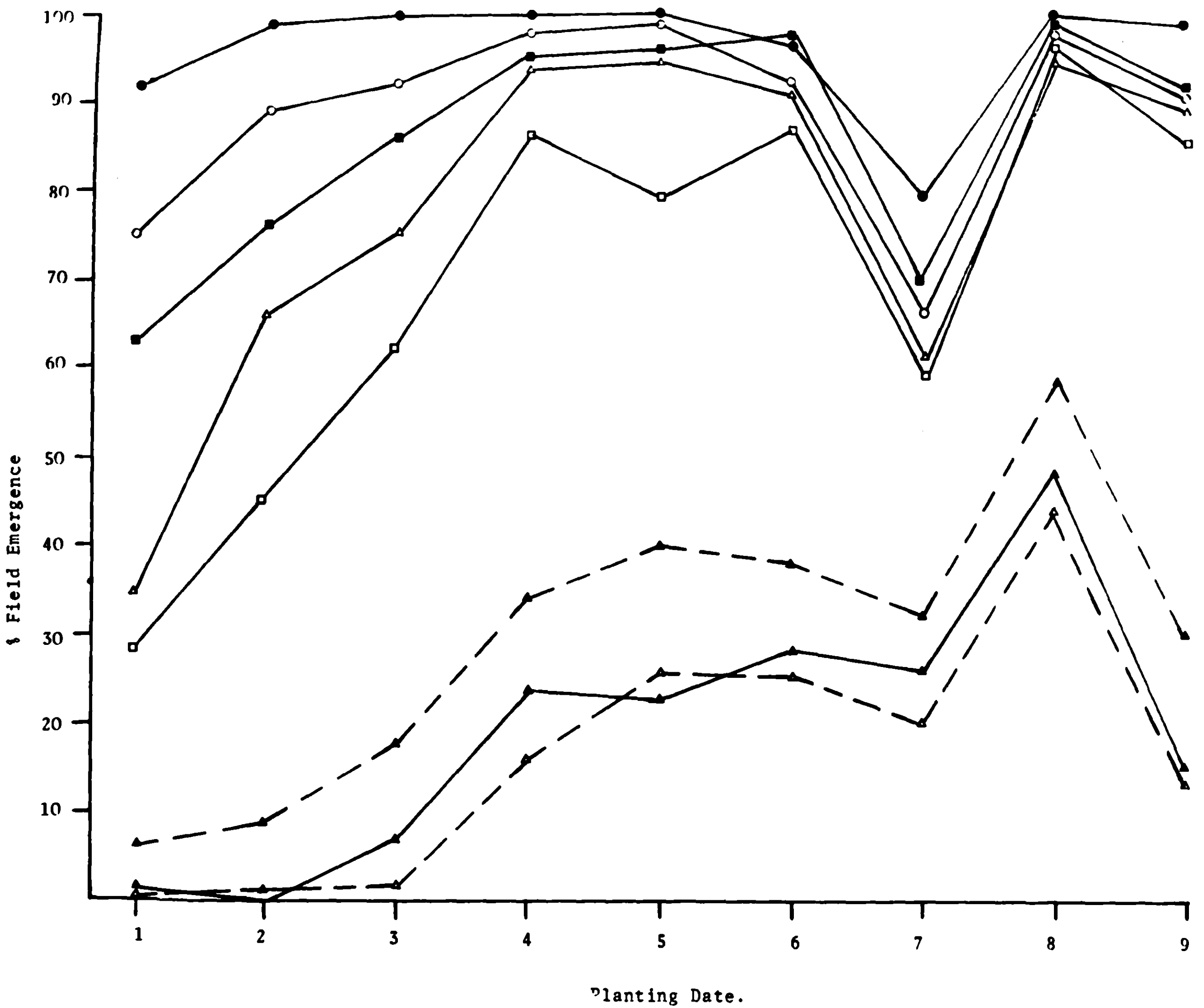

100
90
80
70
60
50
40
30
20
10
% Field Emergence
1
2
3
4
5
6
7
8
9
Planting Date.

Fig. 5. The percentage field emergence of the eight seed lots planted at the Sword site on eight occasions during the 1970 season. Seed lots are shown as follows:

39	● ————————
47	○ ————————
04	□ ————————
54	■ ————————
03	△ ————————
80	▲ ————————
21	△ ——— ——— ———
20	▲ ——— ——— ———

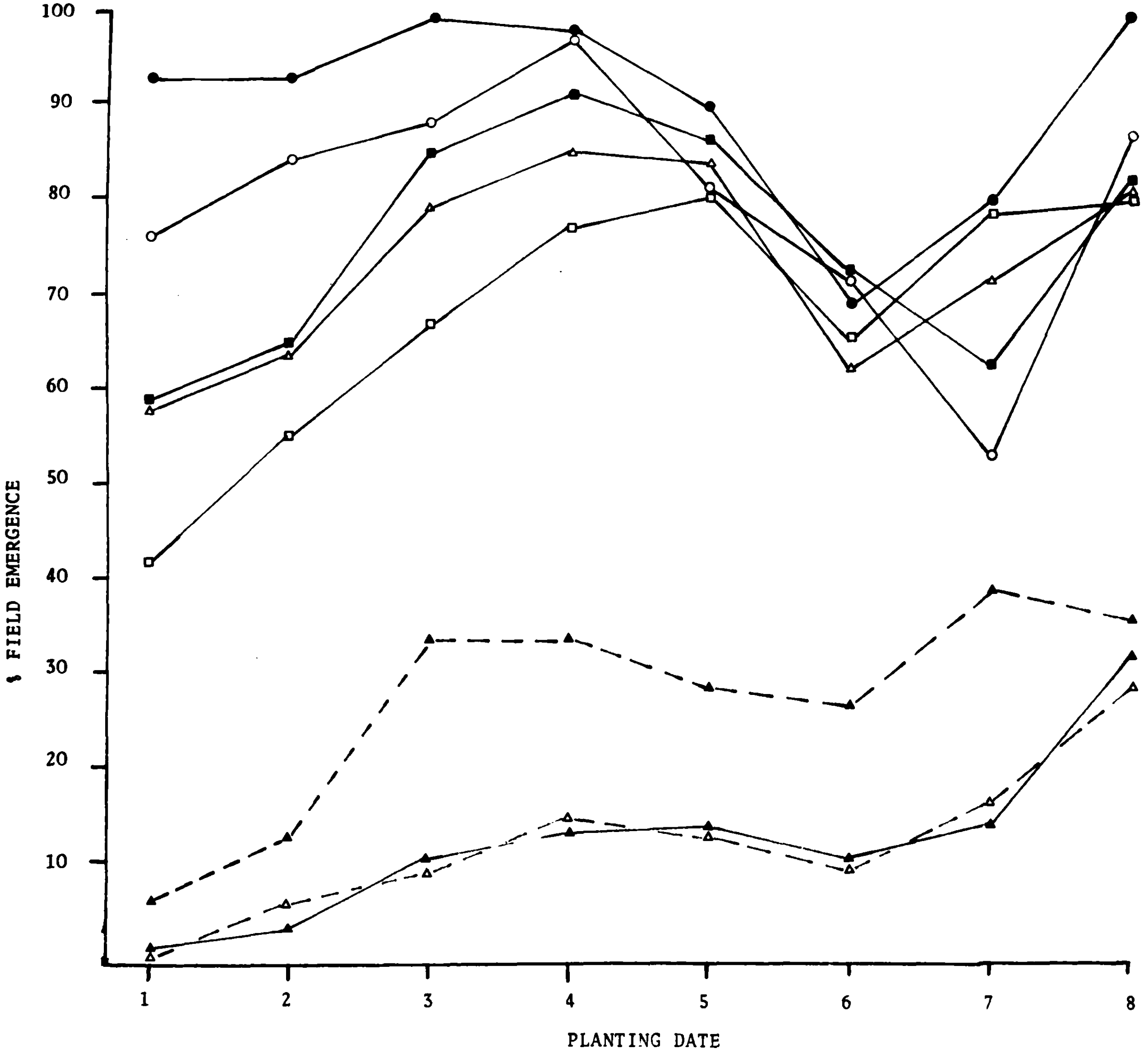
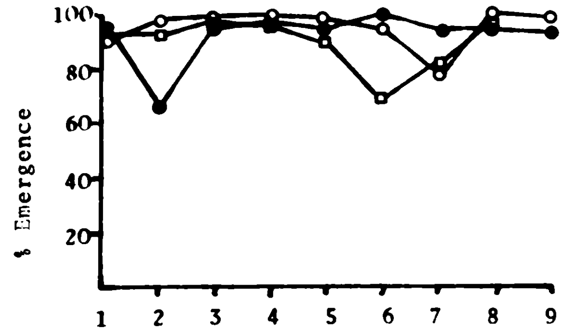
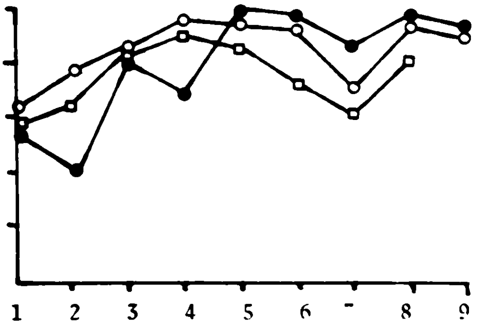

100
90
80
70
60
50
40
30
20
10
% FIELD EMERGENCE
1
2
3
4
5
6
7
8
PLANTING DATE

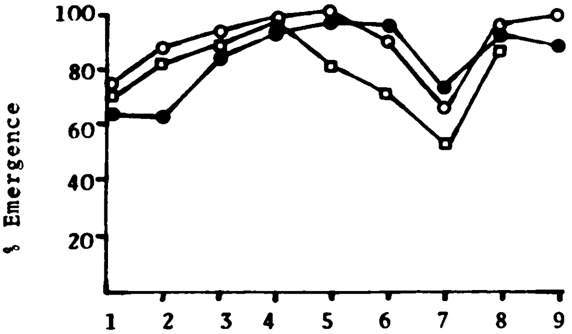
Fig. 6. The percentage field emergence of the eight seed lots at the three planting sites. The three sites are shown as follows: Nursery ● ,Field ○ and Sword □ .



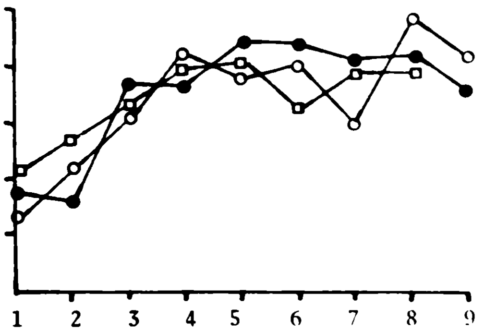
39



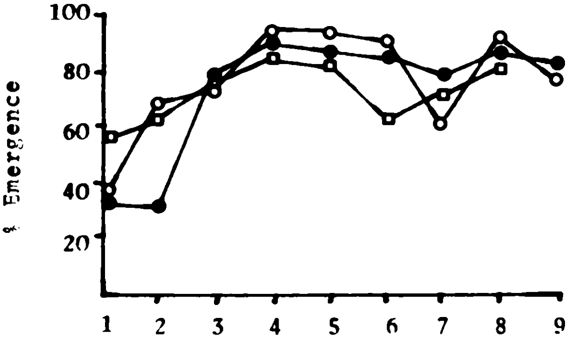
03



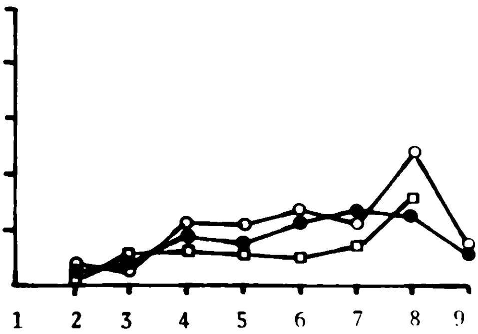
47



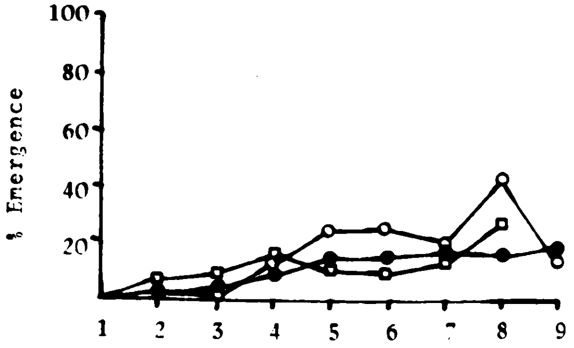
04



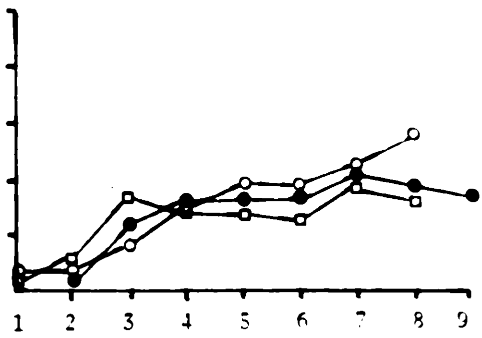
54



80



21



20

Planting Date.

Planting Date.

Seed lots 80, 21 and 20 had markedly lower emergence rates than the other five seed lots, at all the planting dates. Although these poor seed lots did improve slightly at the later planting dates, the highest emergence recorded was 58% by seed lot 20.

All the seed lots showed a lower level of emergence at planting date seven, June 2nd, and reference to Fig. 2 indicates that at that time soil temperatures were relatively high, and there was no rainfall in the week following the planting. These drought conditions reduced the emergence of all the seed lots and for this reason the results of planting date seven were not included in any of the statistical analyses of this section, or those in section IV.

The seed lots exhibited the same emergence patterns at the Sword site, as the Field, but the ultimate emergence of the seed lots was slightly lower. The drop in emergence, as a result of the drought conditions at planting date seven, was slightly larger, suggesting perhaps that the moisture holding capacity of the Sword soil was lower than that of the Field soil. This was confirmed in the laboratory, the water holding capacity against gravity of the Field soil being 34%, whilst that of the Sword soil was 18%.

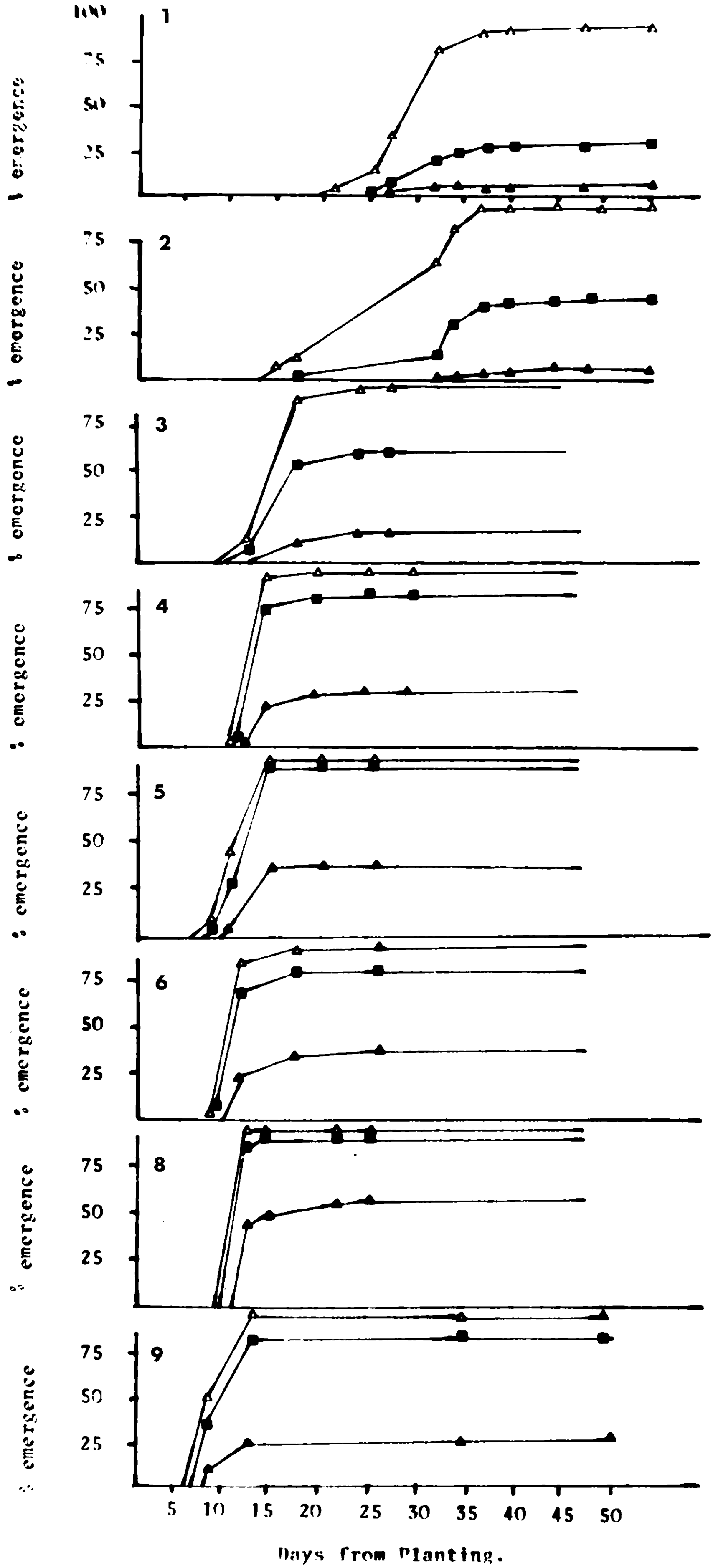
The drop in emergence at planting date seven at the Nursery site was also lower than that at the Field site. The emergence results of planting date two (April 6th), at the Nursery site also had to be omitted from the analysis because of removal of seeds by mice. However, careful inspection of all the sites indicated that only the Nursery site had been affected, and then only at planting date two.

The ultimate emergence of the seed lots indicated that the eight lots could be divided into three categories. Seed lots 39 and 47, achieved high levels of emergence at all planting dates and were termed good. Seed lots 04, 03 and 54, had the ability, during the later planting dates, to achieve high levels of emergence, but exhibited low emergence during the early planting dates. These were termed intermediate seed lots. Seed lots 80, 21 and 20 were classed as poor, since even during the more favourable soil conditions of the later planting dates, they had low levels of emergence.

The emergence of seeds in these experiments can be expressed as curves relating emergence to the time elapsed from the date of planting. Fig. 7 shows the curves for seed lots 39, 04 and 20, and the previously mentioned similarity between the Nursery, Field and Sword results, allowed the curves for these seed lots at the Field site to be used as representative of the response of good, intermediate and poor seed lots, at the three sites.

These curves show that the seed lots emerged more rapidly at the later planting dates. The differences between the different lots in their final levels of emergence are also clearly shown. At the earlier planting dates, particularly one and two, the time taken to emerge and reach ultimate germination was longer than at the later plantings. The good seed lots emerged before the intermediate and poor seed lots at each planting date, but the differences were smaller at the later planting dates. The other striking feature of Fig. 7 is the uniformity of germination time of the good and intermediate seed lots, indicated by the steep slope of their emergence curves, particularly at the later planting dates. The less

Fig. 7. Increase in percentage field emergence of three seed lots of pea with increasing time elapsed after day of sowing for eight planting dates, the seventh planting date was omitted (see text). The three seed lots are shown as follows: 39 (termed good) △ , 04 (termed intermediate) ■ , 20 (termed poor) ▲ .


1
100
75
50
25
% emergence
2
75
50
25
% emergence
3
75
50
25
% emergence
4
75
50
25
% emergence
5
75
50
25
% emergence
6
75
50
25
% emergence
8
75
50
25
% emergence
9
75
50
25
% emergence
5
10
15
20
25
30
35
40
45
50
Days from Planting.

steep slope of the poor seed lot indicated that after the initial seeds had emerged, it took longer to reach its final emergence, than the other two seed lots.

Fig. 8 shows the 50% of ultimate emergence time, estimated for the eight seed lots at the three sites, presented in histogram form. It is noticeable that the speed of emergence increased at the later planting dates. However, at all the planting dates, particularly the earlier ones, seed lots 80, 21 and 20 had a longer 50% of ultimate emergence time than the other seed lots. The drought conditions following planting date seven not only reduced the ultimate emergence of the seed lots, but also decreased their speed of germination as can be seen in Fig. 8.

In Fig. 9 the field emergences of good, intermediate and poor seed lots, 39, 04 and 20 respectively, at the Field site, are plotted against the time the seed lots took to reach 50% of ultimate emergence at each planting date. The ultimate field emergence of the good seed lot was high, irrespective of the time the seed lot took to reach 50% of its ultimate emergence, at each planting date. However, the fluctuations in emergence of the intermediate seed lot, and to a lesser extent, the poor seed lot, were closely associated with the time taken to reach 50% emergence. The highly significant correlation coefficient (0.909; $P < 0.001$) for the intermediate lot, the slightly less significant correlation (0.805 $P < 0.01$) for the poor seed lot, and the insignificant correlation for the good seed lot (0.555 N.S.) reflect the different associations between 50% emergence time and ultimate emergence for the different seed lots.

At the first planting date the intermediate seed lot

Fig. 8. Histograms showing the time taken by the eight seed lots to reach 50% of the ultimate emergence at the three sites on the nine planting dates.

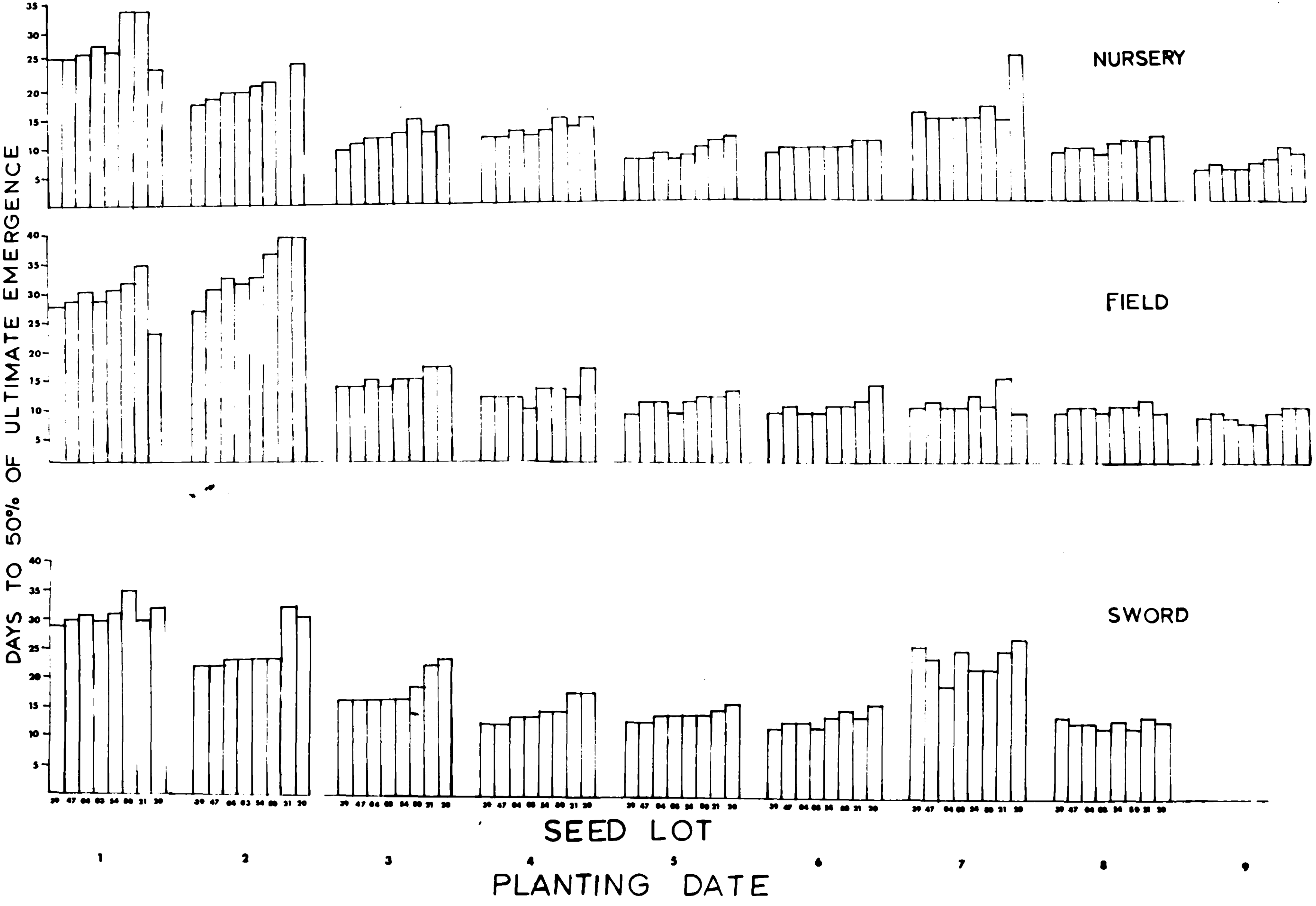
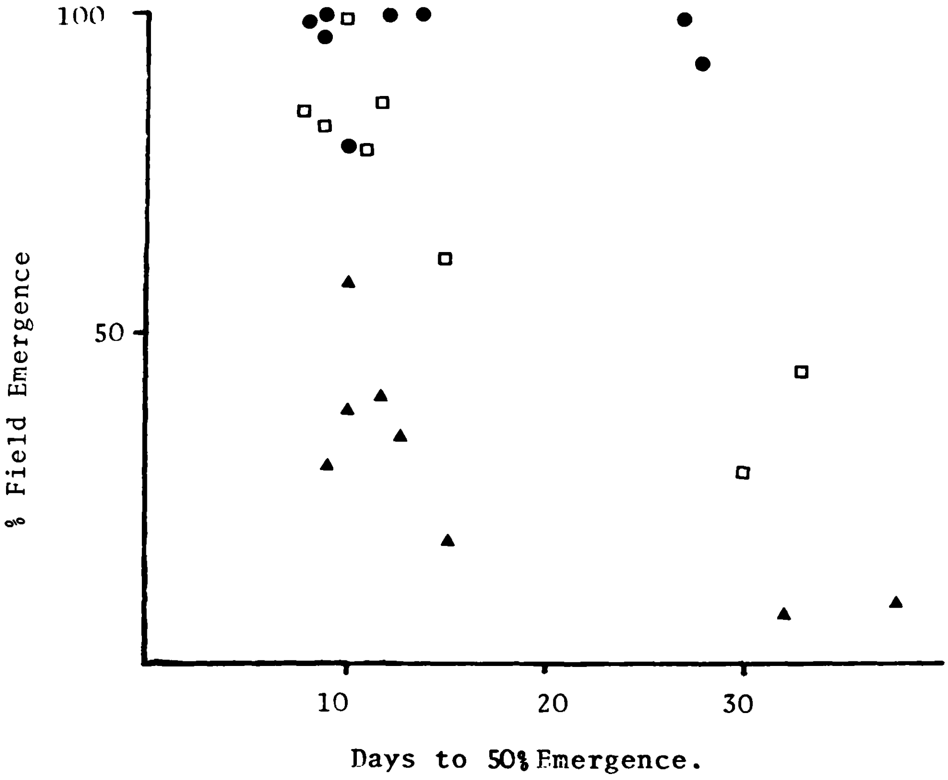
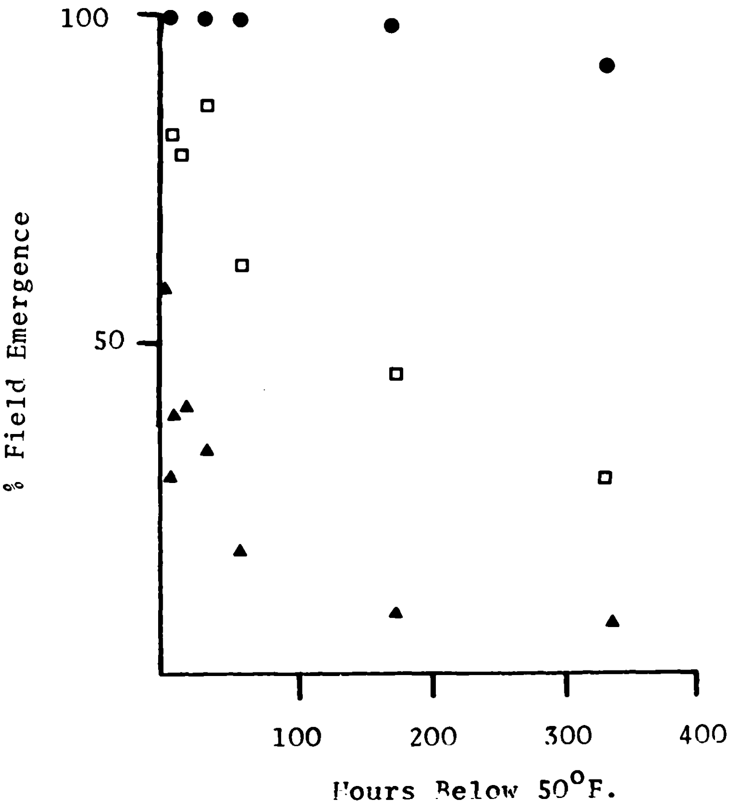

NURSERY
FIELD
SWORD
DAYS TO 50% OF ULTIMATE EMERGENCE
35
30
25
20
15
10
5
40
35
30
25
20
15
10
5
40
35
30
25
20
15
10
5
39 47 04 03 34 80 21 30
39 47 04 03 34 80 21 30
39 47 04 03 34 80 21 30
39 47 04 03 34 80 21 30
39 47 04 03 34 80 21 30
39 47 04 03 34 80 21 30
39 47 04 03 34 80 21 30
39 47 04 03 34 80 21 30
SEED LOT
1
2
3
4
5
6
7
8
9
PLANTING DATE

Fig. 9. The relationship between the time taken to reach 50% of ultimate emergence, and the percentage field emergence, of three seed lots of peas. The three seed lots are shown as follows: 39 (termed good) ●, 04 (termed intermediate) □, 20 (termed poor) ▲.

Fig. 10. The relationship between the number of hours below 50°F two weeks after planting, and the percentage field emergence of the three seed lots of pea. The three seed lots are represented as follows: 39 (termed good) ●, 04 (termed intermediate) □, 20 (termed poor) ▲.


100
50
% Field Emergence
10
20
30
Days to 50% Emergence.

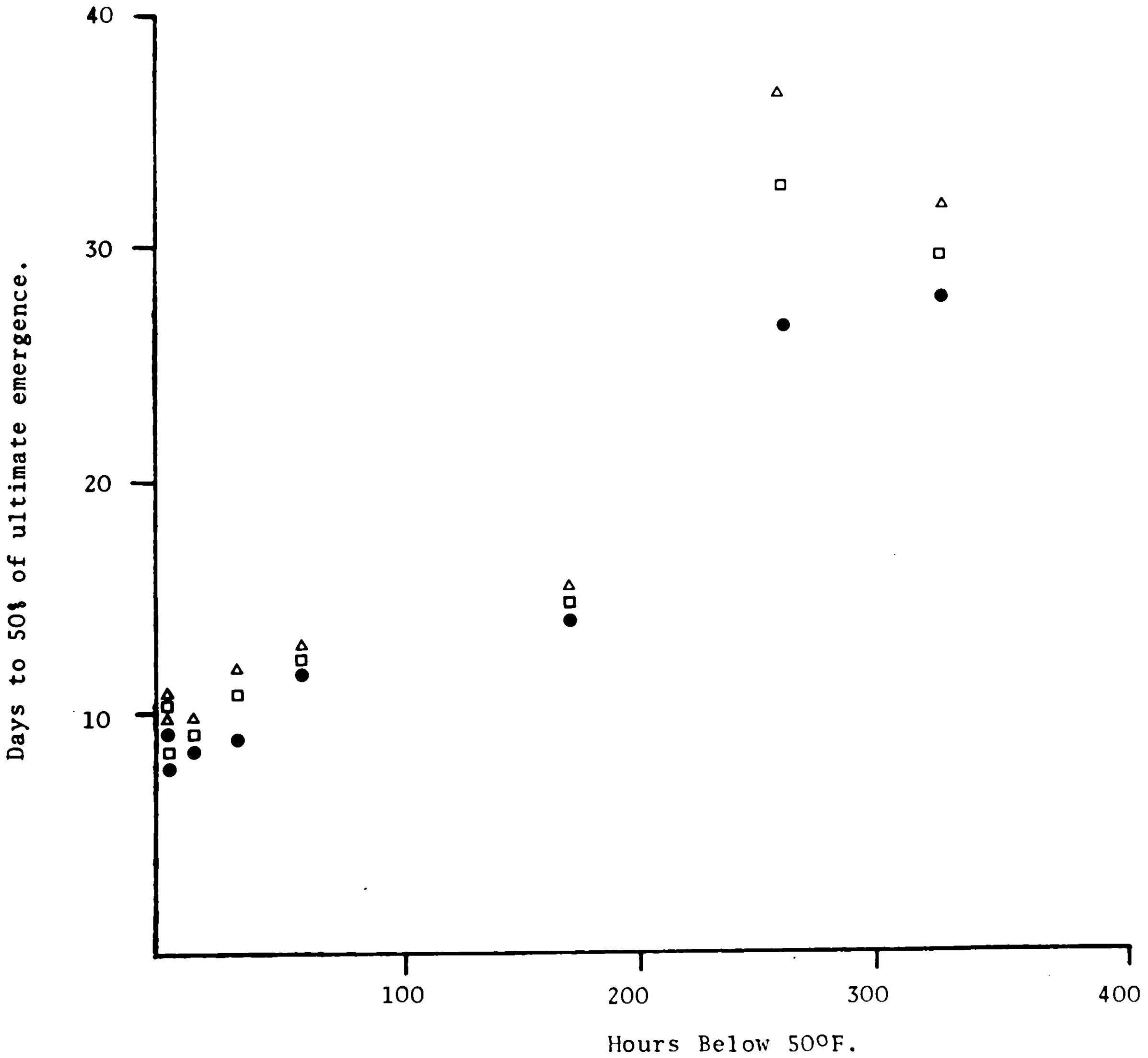

100
50
% Field Emergence
100
200
300
400
Hours Below 50°F.

had an ultimate emergence of 29% after being in the soil thirty days before reaching 50% emergence, but at the last planting date when 50% emergence was reached after ten days the ultimate emergence was 88%. Furthermore, when the ultimate field emergence of the three seed lots was plotted against the accumulated hours below 50°F two weeks after each planting, (Fig. 10) the correlation coefficient again illustrated that the intermediate (0.972; $P < 0.001$) and poor seed lots (0.888; $P < 0.01$) emergences were associated with this temperature measurement. The intermediate and poor seed lots had higher emergences when there were fewer instances of the temperature falling below 50°F after planting. It was clear, therefore, from the last two figures that the length of time the seeds were in the soil before germinating was associated with their ultimate performance in the field, particularly the intermediate seed lot. However, when the time taken to reach 50% of ultimate emergence was plotted against the accumulated hours below 50°F (Fig. 11) it was obvious that the speed of emergence of the three seed lots was influenced in a similar manner by low temperatures.

Low temperatures therefore increased the time in the soil, before ultimate emergence, for all three seed lots. However, only intermediate and, to a lesser extent, the poor seed lots, were influenced in their ultimate emergence by the length of time in soil caused by low temperatures. Hence, temperature appeared to be the most important variable associated with differences in emergence at the different planting dates, but not simply by varying the time taken to emerge.

An overall analysis of the results within each

Fig. 11. The relationship between the number of hours below 50°F in the two weeks after planting and the time taken to reach 50% of ultimate emergence for the three seed lots of peas. The seed lots are shown as follows: 39 (termed good) ● , 04 (termed intermediate) □ , 20 (termed poor) ▲ .



40
30
20
10
Days to 50% of ultimate emergence.
100
200
300
400
Hours Below 50°F.

planting date revealed that the different planting dates did not cause significant differences in emergence, Table 5. This result is surprising considering the response of some of the seed lots to planting date, as shown in Figs. 3 and 5. It probably arose because the good and poor seed lots were relatively unaffected by planting date, and the analysis was unable to detect the differing responses of the intermediate seed lots. A further analysis of the response of the individual seed lots to planting date, Table 6, indicated that in every case except one, the ultimate emergence of the seed lots was significantly influenced by the date on which they were planted.

Table 5. The statistical significance of the influence of the planting date and seed lot on the ultimate emergence at the three sites after analysis of variance on arc sin transformed data.

Source of Variation	Field	Nursery	Sword
Planting date	N.S.	N.S.	N.S.
Seed lot	***	**	**
Interaction	N.S.	N.S.	N.S.

* P < 0.05; ** P <0.01; *** P <0.001

Table 7 shows the mean percentage emergence of viable seeds for three seed lots at the three sites, for the different planting dates. Duncan's Multiple Range Test shows where significant differences in emergence occur. The good seed lot 39, showed some significant differences in emergence due to planting date (Fig. 7), but the largest and most frequent differences occured with the intermediate seed lot 04.

The latter analysis has shown therefore that the good and poor lots were influenced by planting date, but Figs. 3, 4 and 5 and Table 7 indicate that the magnitude of the response was much lower than that of the intermediate seed lots. The good seed lots were able to maintain high levels of field emergence at all the planting dates, whereas the poor seed lots were unable to emerge well even during the more favourable conditions of the later planting dates. The intermediate lots exhibited low emergence at the earlier planting dates where they were subjected to low soil temperatures, but as soil temperature increased at the later planting dates emergence also increased. During the course of the field plantings no periods of heavy rainfall were recorded, and so it was not possible to determine the influence of high soil moisture on emergence in the field.

Table 6. The variance ratios and statistical significance of the response of the individual seed lots to planting dates at the three sites after analysis of variance on arc sin transformed data.

Seed lot	Field	Nursery	Sword
39	1.6 N.S.	3.9 **	5.3 **
47	4.0 **	8.4 ***	7.6 ***
04	36.9 ***	11.8 ***	5.4 ***
03	12.1 ***	21.5 ***	7.2 ***
54	36.4 ***	12.7 ***	8.7 ***
80	29.9 ***	17.2 ***	5.4 **
21	29.9 ***	9.8 ***	11.8 ***
20	27.8 ***	11.3 ***	17.4 **

** $P < 0.01$; *** $P < 0.001$

Table 7. Mean percentage emergence of viable seeds after arc sin transformation of three seed lots at the three sites indicating where significant differences in emergence occur.

Seed	Plant	Field	Nursery	Sword
39	1	69.0 a*	65.75 a*	70.25 b*
	2	74.5 a b	81.5 c	73.75 b
	3	75.75 a b	76.25 b c	72.25 b
	4	77.0 a b	70.5 a b	66.75 b
	5	80.25 b	77.5 b c	53.25 a
	6	71.5 a	73.0 a b c	77.25 b
	7	80.75 b	68.0 a b	
	8	74.25 a b		
04	1	27.25 a*	28.0 a*	36.50 a*
	2	38.25 b	53.0 b c	49.25 b
	3	46.5 c	54.25 b c	54.25 b
	4	67.25 e	60.75 d	56.50 b
	5	54.5 d	65.25 e	48.50 b
	6	56.5 d	58.0 c d	55.25 b
	7	67.5 e	50.5 b	
	8	57.75 d		
20	1	11.75 a*	8.0 a*	12.25 a*
	2	15.5 a	27.25 b	33.75 b
	3	23.25 b	33.5 b	33.0 b
	4	38.75 d	32.75 b	30.0 b
	5	36.75 d	33.0 b	28.75 b
	6	35.5 c d	32.75 b	33.50 b
	7	46.0 e	33.25 b	
	8	29.25 b c		

* Any two means with a letter in common are not significantly different at the $P < 0.05$ level: Duncan's multiple range test.

The indications from this field work were that the variations in emergence of some seed lots of peas were associated with temperature at, and shortly after, planting. The small differences in soil type between the three sites did not influence the emergence of the eight seed lots.

(2) Influence of temperature and moisture on emergence in the laboratory.

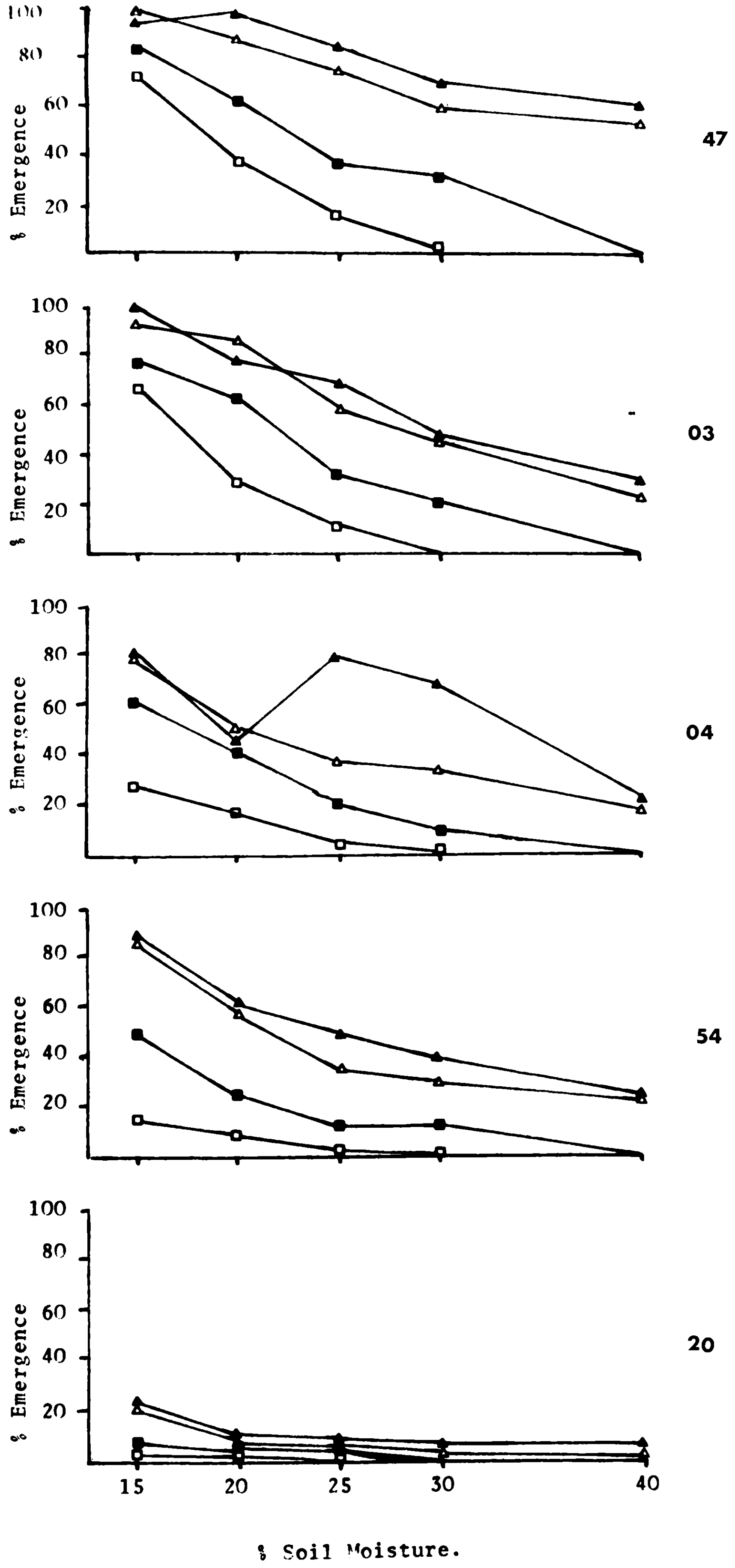
The emergence of the five seed lots of pea were reduced to varying extents by low temperatures and high soil moistures. Ungerminated seeds removed from the soil and planted on water agar revealed infection by Pythium ultimum, indicating an association between the fungus, and pre-emergence mortality of peas.

The results of the tray work are presented in Fig. 12, and although the response patterns are very similar, the magnitude of emergence reductions due to temperature and moisture vary between the different seed lots.

For all the seed lots, emergence at 20°C was greater than at 15°C > 10°C > 5°C. The influence of soil moisture on emergence was also uniform, emergence being greater at 15% than 20% > 25% > 30% > 40%. However, reductions in emergence due to increasing soil moisture were greater at the lower temperatures, particularly for the three intermediate seed lots 04, 03 and 54. The good seed lot 47, showed little reduction in emergence at 15°C and 20°C as soil moisture increased, and even at 5°C it was capable of 71% emergence in the driest soil moisture.

The reductions in emergence of the intermediate seed lots were higher than those of the good seed lot, when soil moisture increased. However, no explanation can be offered

Fig. 12. The percentage emergence of five seed lots of peas, planted in trays of field soil at five different moisture contents and incubated at four temperatures. The incubation temperatures are shown as follows: 20°C ▲, 15°C △, 10°C ■, 5°C □.


% Emergence
100
80
60
40
20
47
03
04
54
20
15
20
25
30
40
% Soil Moisture.

for the large drop in emergence suffered by seed lot 04, in soil of 20% moisture content at 20°C.

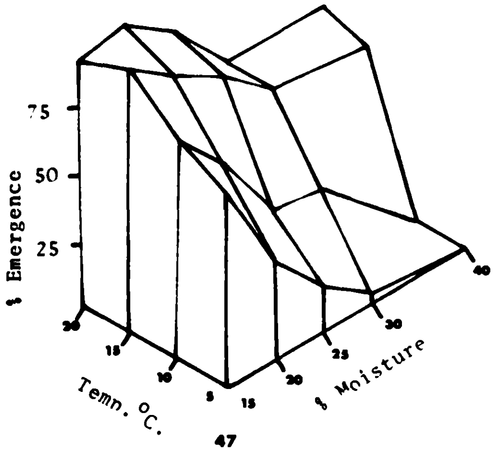
The poor seed lot exhibited very low emergence, and even in the conditions which proved most favourable for the other seed lots, that of 15% soil moisture at 20°C, it had an emergence of only 23%. As the moisture holding capacity against gravity of the soil used in these experiments was found to be 33%, the suitable moisture for emergence, 15%. was substantially lower than the moisture holding capacity of the soil.

The results presented in Fig. 13 show more clearly how emergence of all the seed lots decreased as conditions of low temperature and high soil moisture were approached. The good seed lot, particularly at the higher temperatures, maintained high emergence as the soil moisture increased, but it was unable to do so at the lower temperatures of 5°C and 10°C. The intermediate seed lots did not show this ability, and the poor seed lot is especially obvious in this type of presentation, with its low level of emergence under all conditions.

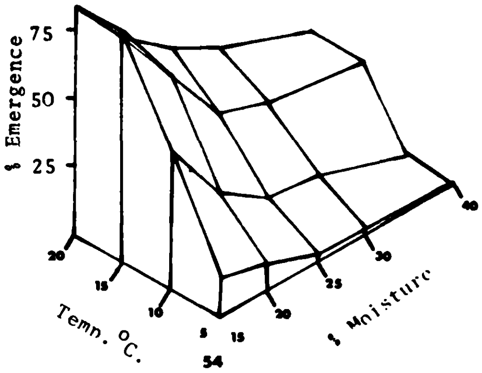
The overall analysis of the influence of seed lot, temperature and soil moisture on emergence, Table 8, indicated that the main effects were all highly significant ($P < 0.001$).

Temperature was confounded in this experiment with time, and was also not replicated, but it was felt that this was unlikely to have any influence on the results of the interactions, temperature x seed lot ($P < 0.001$) was more significant than moisture x seed lot ($P < 0.01$) and the interactions between temperature and moisture was not significant.

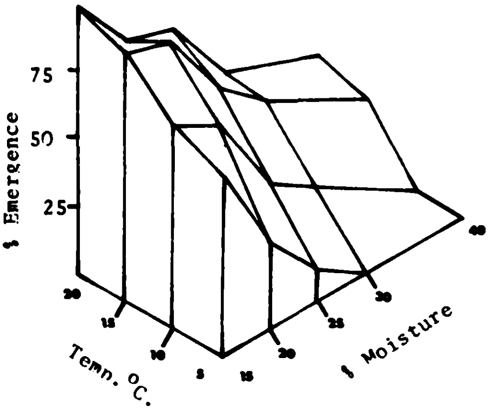
Fig. 13. The influence of soil moisture and temperature on the emergence of five seed lots of peas planted in trays of field soil.


% Emergence
75
50
25
20
15
10
5
Temp. °C.
15
20
25
30
40
% Moisture

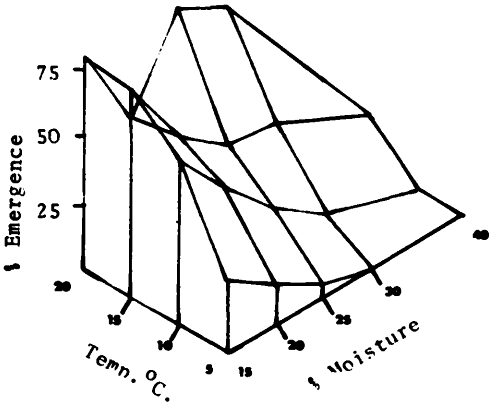
47


% Emergence
75
50
25
20
15
10
5
Temp. °C.
15
20
25
30
40
% Moisture

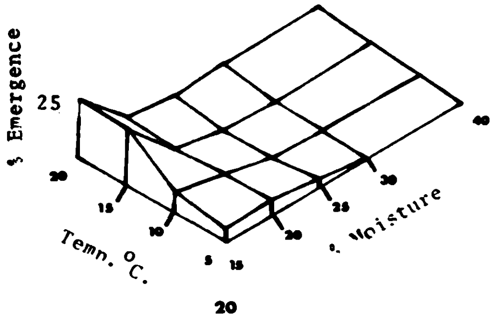
54


% Emergence
75
50
25
20
15
10
5
Temp. °C.
15
20
25
30
40
% Moisture

03


% Emergence
75
50
25
20
15
10
5
Temp. °C.
15
20
25
30
40
% Moisture

04


% Emergence
25
20
15
10
5
Temp. °C.
15
20
25
30
40
% Moisture

20

Table 8. The statistical significance of the influence of seed lot, soil moisture, temperature on emergence and all the possible interactions after analysis of variance of arc sin transformed data.

Source	Significance.
Seed lot	***
Moisture	***
Temperature	***
Moisture x Temperature	N.S.
Moisture x Seed lot	**
Temperature x Seed lot	***
Seed lot x Moisture x Temperature (Error)	

This analysis indicated that temperature was more important in determining the emergence of the seed lots in the trays, than soil moisture but an analysis of the emergence data for each temperature separately revealed that soil moisture had a highly significant influence on emergence, within each temperature (Table 9).

Table 9. The statistical significance of the influence of seed lot and moisture on emergence at each temperature.

	$5^{o}C$	$10^{o}C$	$15^{o}C$	$20^{o}C$
Seed lot	***	***	***	***
Moisture	***	***	***	***
Seed lot x moisture	***	***	**	***

Furthermore, the differences in emergence between the seed lots within one temperature also proved highly significant.

Two points emerge from the tray work. Firstly, both temperature and soil moisture were important in determining the emergence of the seeds, and temperature was the more important of the two. Secondly, the five seed lots tested differed in their responses to changing soil conditions, seed lot 47 showing the highest emergence of the five, and seed lot 20 showing the lowest emergence with seed lot 04, 03 and 54 showing low emergence in the low temperature high moisture treatments, and high emergence in the high temperature, low moisture treatments.

Both in the field and in the laboratory temperature has proved to be important in determining the emergence of pea seeds. Soil moisture, although not shown to be important in the nine field plantings, was found in the laboratory to influence seed emergence. The conditions that lead to the heaviest pre-emergence mortality in the laboratory situation were soils of high moisture at low temperature, and in the field, plantings during low temperature conditions also led to high mortality.

DISCUSSION

These experiments have clearly shown that in conditions of low temperature and high soil moisture some seed lots of peas suffered heavy pre-emergence mortality. Based on their field performances the eight seed lots were classified into three groups; good seed lots, which were capable of high levels of emergence during all conditions, intermediate seed lots, which possessed the ability to produce high levels of emergence, but suffered high levels of pre-emergence mortality under adverse conditions, and poor seed lots which were incapable of producing high levels of emergence in the field.

The laboratory performance in soil for five of the eight seed lots used in the field agreed with the classification based on field performance.

Unfortunately, none of the field plantings were followed by heavy rain, and so the influence of high soil moistures and low soil temperatures in the field, was not investigated. However, the conclusions drawn from the field work agree with those of Harper, Landragin and Ludwig (1955 a) and Wellington (1962) that the largest discrepancies between laboratory germination and field establishment occurred when the period of establishment was prolonged by low temperature. However, an important observation made in this work on peas was that some seed lots could emerge well after a period at low temperature, despite a prolonged period of establishment. The three soil types on which the experiments were sown did not influence the response of the seed lots to low temperature. Harper, Landragin and Ludwig (1955 b) investigating the influence of two soil types, heavy clay and light loan, on the emergence of maize seed, also found that soil type had little effect on seed mortality. They found that differences in mortality were greater between soil samples taken from the same place at different times, than between different soil types.

Harper, Landragin and Ludwig (1955 b) found that the variations in pathogenic potential of the soils were correlated with soil moisture, and concluded that the extent of mortality suffered by maize grains in early spring plantings was not only determined by soil temperature but also by soil moisture. Soil moisture was considered to have a minor influence on pea emergence, but the above mentioned work of

Harper et al, and the work of Hull (1937) who found that rainfall after sowing increased the mortality of pea seeds, suggested that soil moisture was equally important as soil temperature in determining pea emergence. This was confirmed by the emergences of the seed lots of peas in the laboratory. Increases in soil moisture caused substantial reductions in emergence in some seed lots of peas.

Benedict (1969) studying the influence of soil temperature on the development of pea root rot, found that the number of seedlings lost as a result of pre-emergence mortality showed greater variation in relation to pea cultivar, than soil temperature. In this work, the fact that from the field and laboratory work the eight seed lots could be classified into three types according to their field performance indicated that different seed lots within one cultivar differed in their response to adverse soil conditions.

The eight seed lots of pea used in these experiments had differing rates of exudation in the laboratory, and Matthews and Whitbread (1968) and Perry (1967) have shown that seeds with high levels of exudation exhibited more pre-emergence mortality than seeds with low levels of exudation. Hence, the variation in field emergence of the seed lots were probably associated with their differing levels of exudation. However, the exudation rates of the three intermediate seed lots did not prove to be very reliable indicators of their potential field emergences. They had exudation rates similar to those of the good lots and did perform, in the field, like good seed lots late in season, but they exhibited very low levels of emergence when subjected to the low temperatures of the early plantings.

In a discussion about conductivity tests, Perry (1970) suggested it was better to establish grades of vigour, rather than to quote the actual conductivity results, when classifying seed lots. However, the increasing levels of emergence demonstrated by his 'good' seed lots planted at different times in the season, would place them in the intermediate category of the present work. Thus, although this work has demonstrated that the exudation test may not be a completely reliable indicator of potential field emergence, the laboratory work did demonstrate that an alternative test, a type of cold test, may be more accurate.

A 'cold test' for peas was suggested by Clark and Baldauf (1958) in which seeds were planted in field soil at low temperature, then removed to higher temperatures for a few days, before emergence counts were made. Seeds with low emergence potential gave low emergence counts in this test, whereas seeds with high emergence potential gave high counts. The tray treatments of 15% moisture content at 5^oC, in fact separated the intermediate seed lots from the good seed lots, the latter having a higher germination. The poor seed lot had a very low germination in this treatment. This test, therefore, although not identical to the cold test, was capable of distinguishing intermediate from good seed lots, which was an improvement over the exudation test. The cold test has not been extensively used because of the difficulties involved in standardizing the test, but this work has indicated that perhaps more attention should be paid to this type of test, as it may be capable of producing a more accurate classification of seed lots, than the exudation test.

Emergence of seed lots of pea in the field is therefore determined by the interaction of soil moisture, temperature and seed condition. However, the differences in emergence, both between seed lots, and within lots planted in different soil conditions, raise some interesting questions as to the influence of seed condition on field emergence.

High levels of seed exudation have been associated with high pre-emergence mortality (Kraft and Erwin, 1967; Hayman, 1969) but the intermediate seed lots used here, with low levels of exudation, also show high pre-emergence mortality during conditions of high soil moisture and low temperature. Hayman (1969) postulated that the accumulation of exudates around seeds at low temperatures, when the speed of germination was reduced, allowed fungi to increase their inoculum potential around seeds. This may occur in the case of the intermediate seed lots, and eventually the seeds may succumb to pathogenic attack. Another possibility is that soil conditions influence the physiological condition of some seeds, so as to predispose them to fungal attack.

Low temperatures and high soil moistures reduced the emergence of some seed lots of pea, and some of the differences in emergence between seed lots could be accounted for by their differing rates of exudation. However, it is still not clear how soil conditions influence the incidence of pre-emergence mortality, and why some seed lots respond more than others to changes in soil conditions.

SECTION III

PHYSIOLOGICAL RESPONSES OF GERMINATING SEEDS TO MOISTURE AND TEMPERATURE

INTRODUCTION.

Soil conditions of low temperature and high moisture lead to low emergence in some seed lots of peas in the field and in trays of unsterilized soil in the laboratory. The extent of the reduction in emergence, produced by these soil conditions, differs between seed lots, even though the lots have equally high laboratory germinations.

Baylis (1941) and Angell (1952) found that Pythium spp. were responsible for pre-emergence mortality in peas, and that high soil moistures increased the incidence of disease. Soil temperature has also been shown to influence pre-emergence mortality. Schulz and Bateman (1969) found that seeds of pea, bean, cucumber and maize were more heavily infected by Rhizoctonia solani, at low temperatures than at high temperatures.

Soil moisture and temperature may affect the incidence of pre-emergence mortality of seeds, through their influence on the activity of the pathogen, the exudation rates of the seeds or other aspects of the seeds' physiological condition which render the seeds more predisposed to fungal attack.

Low temperatures reducing the growth of the host relatively more than that of the fungus, have been shown by Leach (1947) to lead to increased incidence of disease. Brown & Kennedy (1966) examined the influence of oxygen concentrations on host and pathogen growth rates, and found that under conditions of low oxygen availability, conditions which are likely to occur in soils of high moisture, the growth of soybeans was more restricted than that of the pathogen, Pythium spp.

There is no general agreement concerning the importance of soil moisture, in influencing fungal activity. Griffin (1963) thought it unlikely that fungal activity would be reduced in soils of low moisture content, even below permanent wilting point, and Kerr (1964) working with *Pythium ultimum* confirmed this. Since diseases caused by *Pythium spp*. are more severe in wet soils than dry soils, Garrett (1938) suggested that soil moisture primarily influenced the fungus. Many species of *Pythium* produce zoospores, and thus a wider dispersal of zoospores would be facilitated by these high levels of soil moisture. However, zoospores of *Pythium ultimum*, the species most important in pre-emergence mortality of peas (Flentje, 1964) have never been observed (Middleton, 1943). The increased levels of pre-emergence mortality under conditions of high soil moisture may not therefore be due to the conditions influencing the activity of the fungus.

The association between high seed exudation and heavy seed mortality in unsterilized conditions has been reported for maize (Tatum, 1954), turnip (Barton, 1957), castor bean (Thomas, 1960), French bean (Schroth & Cook, 1964), peas (Flentje & Saksena, 1964), mung bean (Kraft & Erwin, 1967), pine (Agnihotri & Vaartaja, 1968) and cotton (Hayman, 1968). Furthermore, increased fungal spore germination in the presence of seed exudates has been reported by Cook & Flentje (1967)and Schroth & Snyder (1961). Therefore, soil conditions that increase seed exudation may stimulate greater fungal activity, and increase the chances of infection by increasing the inoculum potential around the seed (Garrett, 1938).

Increased loss of sugars by exudation has been shown by Kerr (1964) to occur as a result of increased soil moistures, and Brown & Kennedy (1966) found that exudation from soybean seeds increased under conditions of low oxygen availability. Exudation rates of seeds into steep water have been found to be greatest at low temperatures both for cotton (Hayman, 1969) and for peas (Perry & Harrison, 1970). Since seed lots may differ not only in their initial exudation rates, but also in the way their exudation rates are influenced by changing soil conditions, the differing responses to variations in soil conditions shown by seed lots in the amount of pre-emergence mortality may be due to differences in the response of exudation to conditions.

However, evidence exists indicating that the influence of exudation on fungal infection may not be as straightforward as has been suggested. Cook & Snyder (1965) reported that although the exudates of bean seeds stimulated the germination of <u>Fusarium</u> <u>solani</u> spores, lysis of germ tubes occurred shortly after spore germination. Flentje & Saksena (1964) found that hand-harvested pea seeds artificially damaged to increase their exudation rates to the level of mechanically harvested seeds, exhibited less pre-emergence mortality than the mechanically harvested seed. Thus, although the association between high seed exudation and pre-emergence mortality is well established, there is some doubt about differential exudation being the main factor responsible for differences in pre-emergence mortality between lots.

The influence of soil conditions on the physiology of seeds has received little attention in the past. Woodstock & Pollock (1965) found that seeds of lima bean exposed to low temperatures during imbibition, suffered a reduction in respiration, and Sherwin & Simon (1969) found increased levels of lactic acid and ethanol in bean seeds germinated under wet conditions.

Much of the work on the influence of soil conditions on the physiology of plants has been done on roots. Crawford (1966) examined the anaerobic respiration of roots of Senecio spp. intolerant of flooding and found that their rates of glycolysis increased in response to anaerobic conditions after a period of growth in conditions of high moisture. This increase did not occur in plants tolerant of flooding. Flooding induced high levels of alcohol dehydrogenase activity (Crawford, 1967) in intolerant plants, and subsequently large amounts of ethanol accumulated in their roots.

It has been suggested that death of some intolerant plants when left in flooded soil conditions resulted from the accumulation of toxic levels of ethanol. Furthermore, Bolton & Erickson (1970) working with tomatoes found that increased levels of ethanol in the sap were associated with increased levels of oxygen stress at the roots. Under conditions of oxygen stress therefore plant roots normally accumulate ethanol, but Crawford (1969) has demonstrated that plants from wet habitats withstand flooding by being able to respond metabolically to the conditions without adversely affecting their growth.

An investigation of the respiratory and ethanol accumulation rates of seeds may indicate not only that basic differences occur between seed lots, but also that seed lots may respond differently to conditions of high moisture. Matthews (1971) found that the condition of the cotyledons, particularly how readily they were infected by Pythium spp., was an important feature of seeds predisposed to pre-emergence mortality. However, no work has been done on the influence of soil conditions on the readiness with which the cotyledons of seeds become infected, and how different seed lots respond to these conditions.

The purpose of the work of this section was to examine the influence of conditions of high moisture and low temperature on the respiration and exudation of different seed lots of peas, and to determine the effect of those conditions on predisposition to pathogenic attack.

MATERIALS AND METHODS

In the respiration and exudation experiments six seed lots of the cultivar Dark Skinned Perfection were used. All had high laboratory germination, but differed in their field emergences (Table 2). On the basis of their field performance they were divided into three groups; 39 & 47 termed good with high emergence in most field plantings; 04, 03 & 54 termed intermediate with emergences which varied markedly according to soil conditions, and 20 termed poor with low emergence at all field sowings.

The predisposition experiments were conducted on intermediate seed lot 54, poor seed lot 20 but due to the shortage of seeds of seed lots 39 & 47, a new seed lot 73, was used as a representative of a good seed lot.

Plastic columns, 3" diameter were cut into sections 3", $4\frac{1}{2}$"; 6" and 9" long, and placed in 3" deep enamel trays. The top one inch of each column, which was detachable, was held in place by autoclave tape. Graded fine sand, washed in deionized water and dried at 100°C for 72 hours was used to fill all the columns, and deionized water was maintained at a depth of 1" in the enamel trays, throughout the duration of an experiment. The moisture contents, expressed as a percentage of the weight of the oven dry sand, in the top inch of the 3", 4½" 6" and 9" columns were 22%, 17% 11% and 4% respectively.

The columns were left to equilibrate for 24 hours before being used in an experiment. Ten seeds were placed in each treatment column, each seed having previously been subjected to 24 hours in a six inch column at 20°C. Experiments conducted at 5°C and 20°C both had this pretreatment to ensure that all the seeds used were at the same stage of germination at the time of treatment. The seeds were removed from the treatment column after 24 hours.

(1) Respiration Studies

(a) Gaseous Exchange.

The seeds were washed free of sand in distilled water and three replicate flasks, each containing four seeds and 2 ml of distilled water, were then prepared on the Gilson Differential Respirometer. After thirty minutes equilibration the oxygen uptake and carbon dioxide evolution of the seeds was measured. (Section I and Appendix I).

The experimental samples were then dried at 70°C for 72 hours to determine their dry weights.

(b) Ethanol Accumulation.

Forty seeds, obtained from four identical treatment columns were homogenized in an MSE Atomix for one minute with 50 ml of iced distilled water. The homogenate was then filtered, and in the short time before they were used, the filtrates were stored over ice. The filtrates were assayed for ethanol by injecting 2 ml samples into a Perkin-Elmer gas chromatograph equipped with an integrating recorder. The partition column consisted of Carbowax 100 on AW-DMCS Chromosorb W. The concentration of ethanol was read by comparing the peak heights and integrated areas of the samples, with those of known concentration of ethanol (Appendix II).

(2) Exudation.

The top inch of each treatment column was removed and placed in a beaker, after the seeds had been removed. Deionized water was added until the total amount of water present in each beaker was 200 ml. This involved the addition of 119 ml, 132 ml, 150 ml, and 189 ml, of water to the sand from the 3", 4½", 6", and 9" columns respectively. After stirring, the conductivity of the water was measured using a Portland Conductivity Meter.

Every alternate experiment contained several columns in which no seeds were planted, but the conductivity of the top inch of sand was again measured, so that any changes in the basic conductivity of the sand would be recorded.

A further experiment was also conducted on two intermediate seed lots 54 and 04. After the seeds were removed from the treatment columns, they were placed in 6" columns for a further 24 hours and the conductivity of this sand was measured. This experiment was conducted to determine if the seed lots placed in the 6" columns for the second time had exudation rates appropriate to that substrate moisture, or whether their exudation rates were those that had been adjusted in the treatment columns.

(3) Nitrogen Gassing.

A small experiment was conducted on seed lots 39, 04, 03, 54, and 20 without them having been subjected to the pretreatments or treatment columns.

After 24 hours imbibition in petri dishes on filter paper, 10 seeds per dish with 16 ml of water, the oxygen uptake and carbon dioxide evolution of the seed lots was measured using Gilson No.5 all-glass manometers. The respirometers were then filled with nitrogen gas for thirty minutes, the seed lots being subjected to completely anaerobic conditions during that time. At the end of that time the carbon dioxide evolution of the seeds was measured under anaerobic conditions.

(4) Predisposition.

Seeds of each seed lot were subjected to each individual moisture treatment at 5°C and 20°C and following the treatments, the seeds were removed from the columns, some being planted in trays of field soil at 25% moisture content, others being planted in trays of sterile sand at 11% moisture content. Each tray held forty seeds, ten from each different treatment column. The four groups of seed from the columns were sown randomly in the trays, in two rows of five seeds each. After planting, the trays were covered with moisture-proof wrap, and incubated at 10°C. After 48 hours, one tray of field soil was removed and fungal isolations were performed on the seeds, leaving the four other trays of field soil undisturbed to obtain the subsequent germination of the seed lots after their various pretreatments. Fungal isolations were done by washing soil from seeds with tap water, removing the testa and washing the cotyledons and embryo axis in three changes of sterilised distilled water. Each cotyledon was cut into four pieces and the axis and cotyledon pieces from each seed were then placed separately on plates of tap water agar and incubated at 20°C.

The seeds planted in sterile soil were control seeds, used to obtain the germination capacity of the seeds in sterile conditions after the different pretreatments. These trays were also incubated at 10°C, emergence being counted until no further emergence occurred.

RESULTS

(1) Respiration Studies.

The oxygen uptake and carbon dioxide evolution results are presented as µl gas/h.g. dw. of seed. From the data it was also possible to calculate the R.Q. values of the different seed lots subjected to the different moisture and temperature treatments. The results given in the Tables and Figures are the means of six replicates for experiments conducted at 20°C, and three replicates for experiments conducted at 5°C.

(a) Oxygen Uptake.

The oxygen uptake values of the six seed lots are presented in Tables 10(a) and 10(b) and Fig. 14. Duncan's Multiple Range Test has been applied to the results in Tables 10(a) and 10(b).

Fig. 14. The mean oxygen uptake for six seed lots of peas, measured in air, after 24 hours in four different substrate moistures, shown as percentage moistures of oven dry sand, at 5°C ■ , and 20°C □ .

Fig. 15. The mean carbon dioxide evolution for six seed lots of peas, measured in air, after 24 hours in four different substrate moistures, shown as percentage moistures of oven dry sand, at 5°C ■ , and 20°C □ .

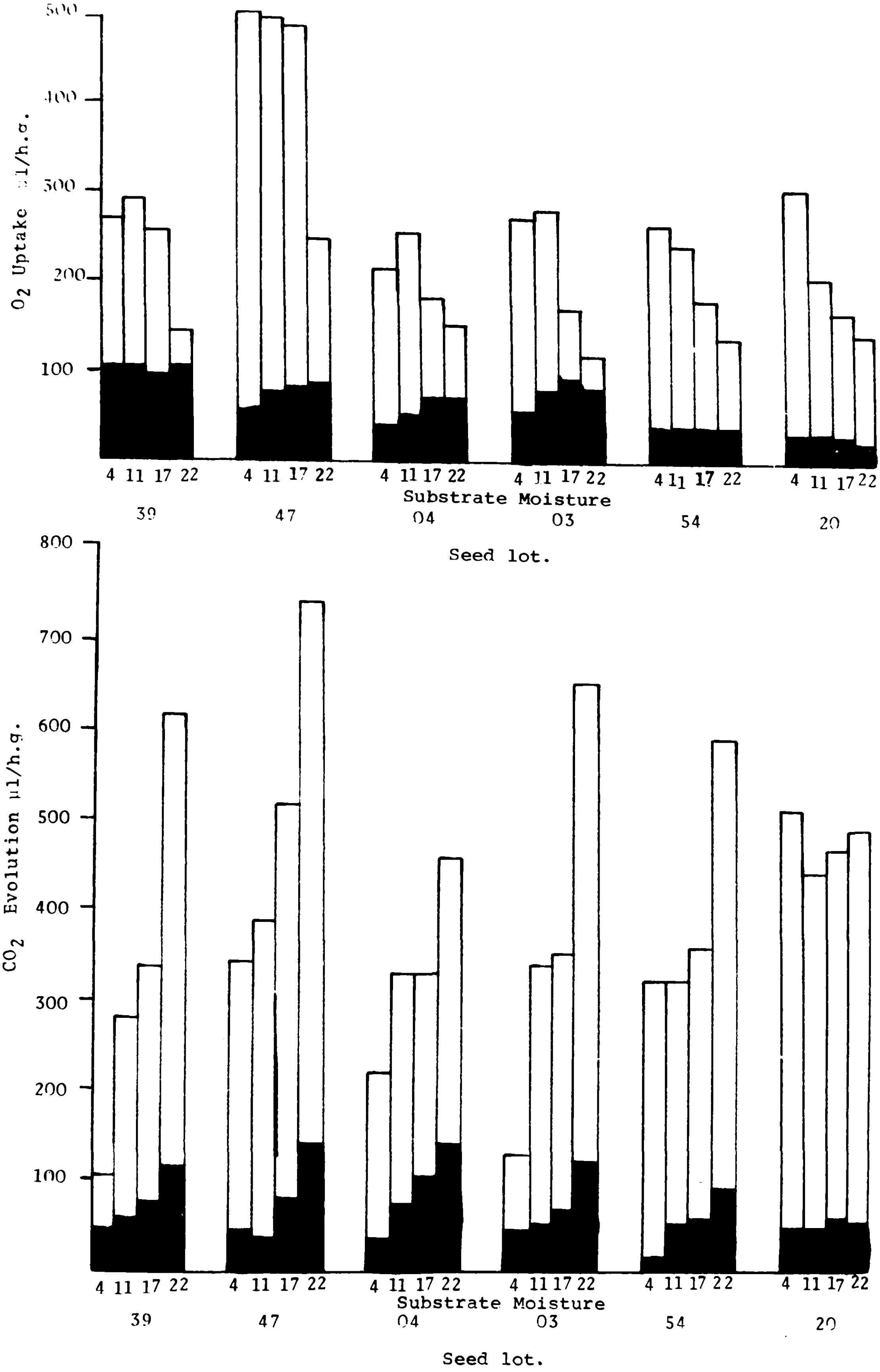

O2 Uptake µl/h.g.
500
400
300
200
100
4 11 17 22
Substrate Moisture
39
47
04
03
54
20
Seed lot.
CO2 Evolution µl/h.g.
800
700
600
500
400
300
200
100
4 11 17 22
Substrate Moisture
39
47
04
03
54
20
Seed lot.

Table 10. Mean oxygen uptake values of replicates of four seeds, for the six seed lots subjected to the 4 moisture treatment at 5°C (a) and 20°C (b) expressed as µl/h.g. dw.

(a) 5°C

		Seed Lot				
Moisture	39	47	04	03	54	20
22%	104	84	65	80	36	22 *
17%	95	82	69	90	36	31
11%	103	77	52	78	38	28
4%	104	54	42	55	35	31

(b) 20°C

		Seed Lot				
Moisture	39	47	04	03	54	20
22%	130	240	149	111	133	142 *
17%	255	450	183	167	180	166
11%	291	494	257	270	240	196
4%	270	493	209	282	263	301

* A continuous line join means not significantly different from one another at $p < 0.05$

At 5°C the differences in uptake between the seed lots were quite pronounced, the good seeds having higher uptakes than the intermediate and poor seed lots. However, at this temperature, substrate moisture had very little effect on the oxygen uptake values, Duncan's Multiple Range test indicating that only seed lots 47 and 03 were in any way influenced, and even then the response was to increase their uptakes slightly as the moisture increased.

Substrate moisture had a greater influence at 20°C. Table 10(b) indicates that the oxygen uptakes of the two good seed lots were not reduced until they were subjected to the wettest substrate moisture, 22%. The three intermediate seed lots were only able to maintain high uptakes in the 4% and 11% treatments, above that their oxygen uptakes were reduced. The uptake of the poor seed lot was reduced by increasing substrate moisture above 4%, indicating the profound effect substrate moisture had on the respiration of that particular seed lot.

(b) Carbon Dioxide Evolution.

The mean evolution figures for the six seed lots subjected to the various treatments are presented in Tables 11(a) and 11(b) and in histogram form in Fig. 15.

Table 11. Mean carbon dioxide evolution values of replicates of four seeds, for the six seed lots subjected to the four moisture treatments at 5°C(a) and 20°C(b) expressed as μl/h.g. dw.

(a) 5°C

	Seed Lot					
Moisture	39	47	04	03	54	20
22%	111	137	137	120	94	55
17%	74	78	107	69	58	59
11%	58	36	73	52	54	50
4%	47	47	33	47	14	50

(b) 20°C

	Seed Lot					
Moisture	39	47	04	03	54	20
22%	606	882	460	649	590	493
17%	335	533	328	353	358	478
11%	270	388	331	342	338	432
4%	106	438	211	132	325	460

The output of carbon dioxide at 5°C was considerably smaller than at 20°C. The carbon dioxide evolution of the poor seed lot, one of the intermediate seed lots and one of the good seed lots, were unaffected by substrate moisture. Although the other seed lots did show increases in evolution as the substrate moisture increased, the increases were not large.

At 20°C the seed lots exhibited increases in carbon dioxide evolution with increasing moisture. The poor seed lot, distinguished from the other lots by its higher level of evolution, was again unaffected by substrate moisture. The two good seed lots, and one intermediate seed lot (03), showed very large increases in carbon dioxide output between the 17% and 22% moisture content treatments. The other two intermediate seed lots showed less pronounced increases in output as the moisture level rose.

(c) Respiratory Quotient.

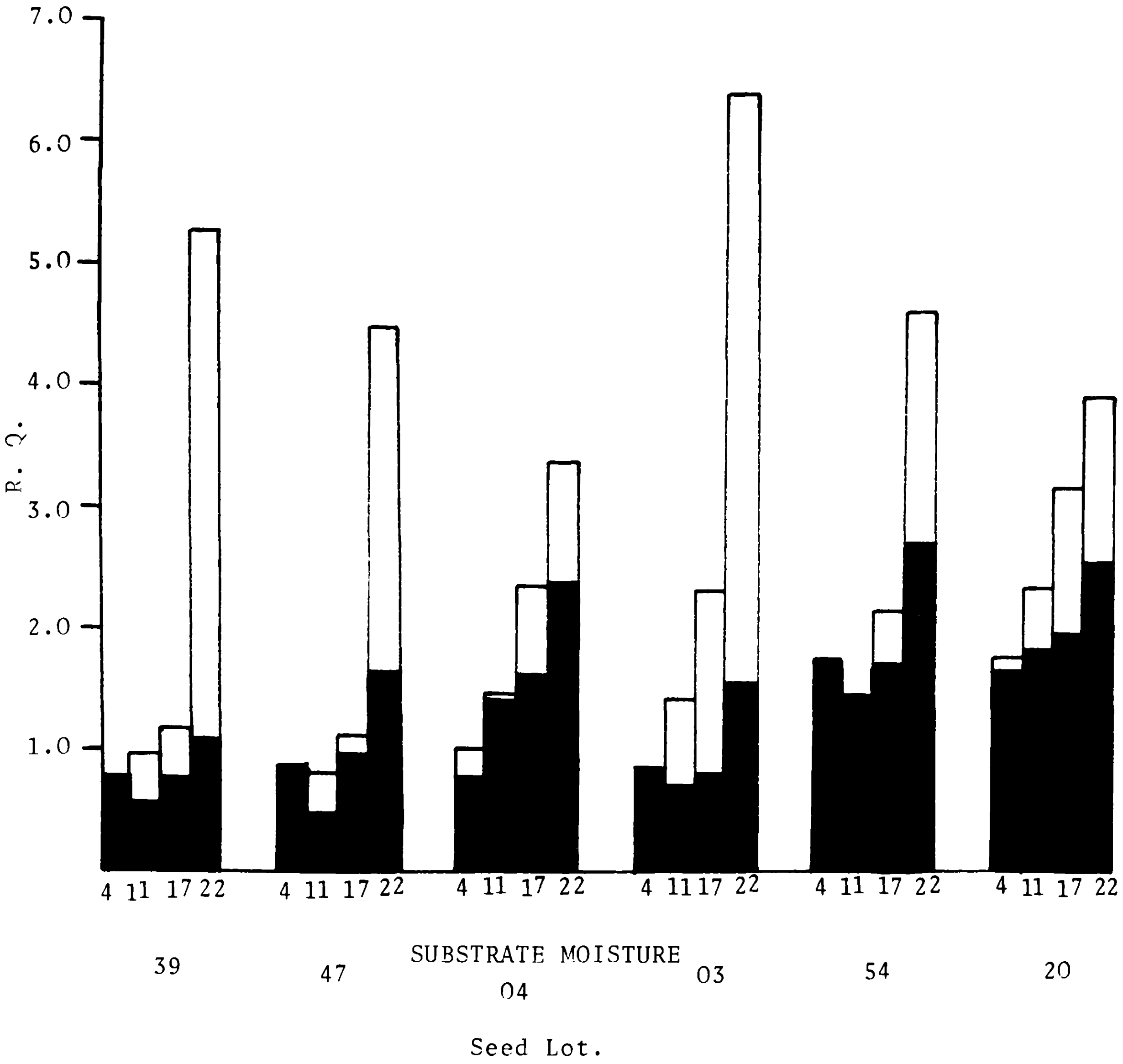
The R.Q. values, calculated from the oxygen uptake and carbon dioxide evolution figures of the six seed lots, previously given are presented in Tables 12(a) and 12(b) and Fig. 16.

Table 12. Mean R.Q. values of replicates of four seeds, for the six seed lots subjected to the four moisture treatments at 5°C(a) and 20°C(b).

(a) 5°C

	Seed Lot 39	47	04	03	54	20
22%	1.03	1.64	2.34	1.53	2.68	2.52
17%	0.77	0.96	1.60	0.77	1.68	1.94
11%	0.56	0.47	1.45	0.68	1.46	1.82
4%	0.75	0.88	0.77	0.86	1.75	1.63

Fig. 16. The mean respiratory quotients for six seed lots of peas, measured in air, after 24 hours in four different substrate moistures, shown as percentage moistures of oven dry sand at 5°C ■, and 20°C □.



7.0
6.0
5.0
4.0
3.0
2.0
1.0
R. Q.
4 11 17 22
4 11 17 22
4 11 17 22
4 11 17 22
4 11 17 22
4 11 17 22
SUBSTRATE MOISTURE
39
47
04
03
54
20
Seed Lot.

(b) 20°C

	Seed Lot					
	39	47	04	03	54	20
22%	5.29	4.66	3.32	6.65	4.55	3.84
17%	1.14	1.14	2.33	2.31	2.16	3.19
11%	0.93	0.79	1.44	1.42	1.44	2.34
4%	0.64	0.74	0.99	0.76	1.32	1.17

At 5°C the values were much lower than at 20°C and the two good seed lots had lower R.Q. values than the other seed lots. At 5°C seed lot 39, maintained a low R.Q. about one, at all the moisture treatments, the other good lot did increase its R.Q. value slightly as moisture increased, but only to 1.65. The intermediate and poor seed lots had higher R.Q. values, and the intermediate lots showed significant increases in R.Q. between the 17% and 22% moisture treatments at this temperature.

The differences between the seed lots in their response to increasing substrate moisture were more striking at 20°C than at 5°C. The two good seed lots maintained low R.Q. values, indicating aerobic pathways of respiration until they were subjected to the 22% moisture treatment, when their R.Q. values increased tremendously indicating a powerful anaerobic respiration. The intermediate seed lots did not show such a pronounced response to substrate moisture, except lot 03, which had the highest field emergence of that group. They did, however, show small increases in R.Q. at each increase in substrate moisture. The poor seed lot responded to increases in substrate moisture with small increases in R.Q., lower than those of the intermediate and good seed lots.

At 5°C, therefore, good seed lots maintained aerobic pathways of respiration during the different moisture treatments, whereas at 20°C, they adopted vigorous levels of anaerobic respiration when subjected to the highest moisture treatment. The intermediate and poor seed lots did not in general respond so markedly to increases in substrate moisture, but at the lower moisture levels they did have higher R.Q. values than the good seed lots.

Seeds with high field emergence during adverse conditions were able to maintain high levels of oxygen uptake, until subjected to very high substrate moisture in the laboratory, when they adopted vigorous levels of anaerobic respiration. Seeds with low field emergence were not only incapable of maintaining high levels of oxygen uptake during conditions of high substrate moisture, but were also incapable of increasing their levels of anaerobic respiration greatly.

(2) Ethanol Accumulation.

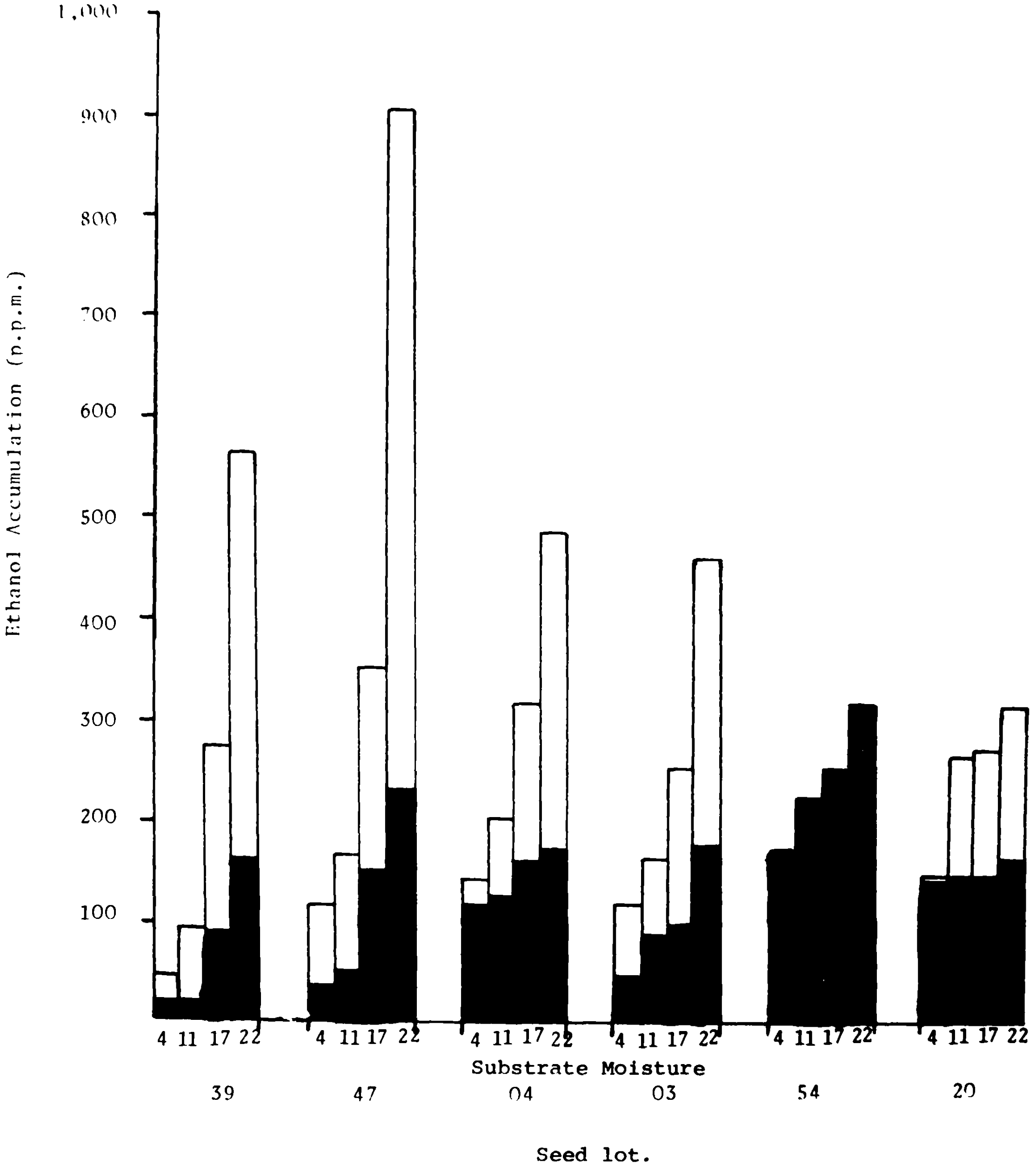
The levels of accumulation of ethanol for the six seed lots are presented in Tables 13(a) and 13(b) and in histogram form in Fig. 17.

Table 13. Ethanol accumulation (p.p.m.)/10gm dw. seed for the six seed lots subjected to the four moisture treatments at 5°C(a) and 20°C(b)

(a) 5°C

	Seed Lot					
Moisture	39	47	04	03	54	20
22%	158	232	171	178	313	109
17%	88	149	162	93	302	95
11%	22	49	123	85	224	95
4%	22	34	114	43	168	95

Fig. 17. Ethanol levels accumulated in seeds of six seed lots of peas after 24 hours in four different substrate moistures, shown as percentage moistures of oven dry sand, at 5°C ■ , and 20°C □ .


1,000
900
800
700
600
500
400
300
200
100
Ethanol Accumulation (p.p.m.)
4 11 17 22
4 11 17 22
4 11 17 22
4 11 17 22
4 11 17 22
4 11 17 22
Substrate Moisture
39
47
04
03
54
20
Seed lot.

(b) 20°C

	Seed Lot					
Moisture	39	47	04	03	54	20
22%	574	903	484	461	263	308
17%	273	349	314	451	223	269
11%	95	164	202	158	100	263
4%	45	115	139	115	59	137

The amounts of ethanol accumulated at 5°C were less than those at 20°C. At 5°C the different substrate moistures had little effect on the accumulation in seed lot 20, but the intermediate seed lots did show increases in accumulation as the moisture level increased. The two good seed lots showed very large increases when they were subjected to the wettest substrate moisture. At 20°C this increase at the wettest substrate moisture was even more striking than at 5°C for the two good seed lots.

Table 14 illustrates the ratio of ethanol found at the wettest moisture against that found at the driest moisture for the two temperatures tested. The poor seed lot showed very little increase in ethanol production at the 22% moisture level, compared to that at the 4% moisture level. The good seed lots 39 and 47, showed marked increases in production at the wettest treatments, the increases being larger at 20°C than at 5°C. The response of the intermediate seed lots lay between those of the good and poor seed lots. Statistical analysis of these results was not possible as those particular experiments were not replicated.

Table 14. The ratio between the ethanol accumulation rates of the seeds at the 22% moisture level, compared to the 4% moisture level.

	Seed Lot 39	47	04	03	54	20
5°C	7.18	6.82	1.50	4.02	1.86	1.14
20°C	12.75	7.85	3.48	4.08	4.44	2.24

The relationship between ethanol accumulation and oxygen uptakes of the seed lots, shown in Fig. 18 was not significant although there was a tendency for the levels of ethanol accumulation to be lower in seeds exhibiting high rates of oxygen uptake. However, the relationship shown in Fig. 19 between ethanol accumulation and carbon dioxide evolution was significant ($P < 0.001$). The accelerated anaerobiosis of some of the seed lots, indicated by their high carbon dioxide evolutions, was therefore accompanied by a high level of ethanol accumulation. The relationship between the R.Q. values of the seed lots and their rates of ethanol accumulation, Fig. 20, was also highly significant ($P < 0;001$) again illustrating that high levels of anaerobic respiration were accompanied by high levels of ethanol accumulation.

(3) Gaseous Exchange in a Nitrogen Atmosphere.

The carbon dioxide output in µl gas/h.g. dw in air was compared to the output when the seeds were subjected to anaerobic conditions in 100% nitrogen gas, and the results are shown in Table 15.

Fig. 18. The relationship between the level of ethanol accumulation, and the rate of oxygen uptake of the six seed lots of peas, after 24 hours in four substrate moistures at 20°C.

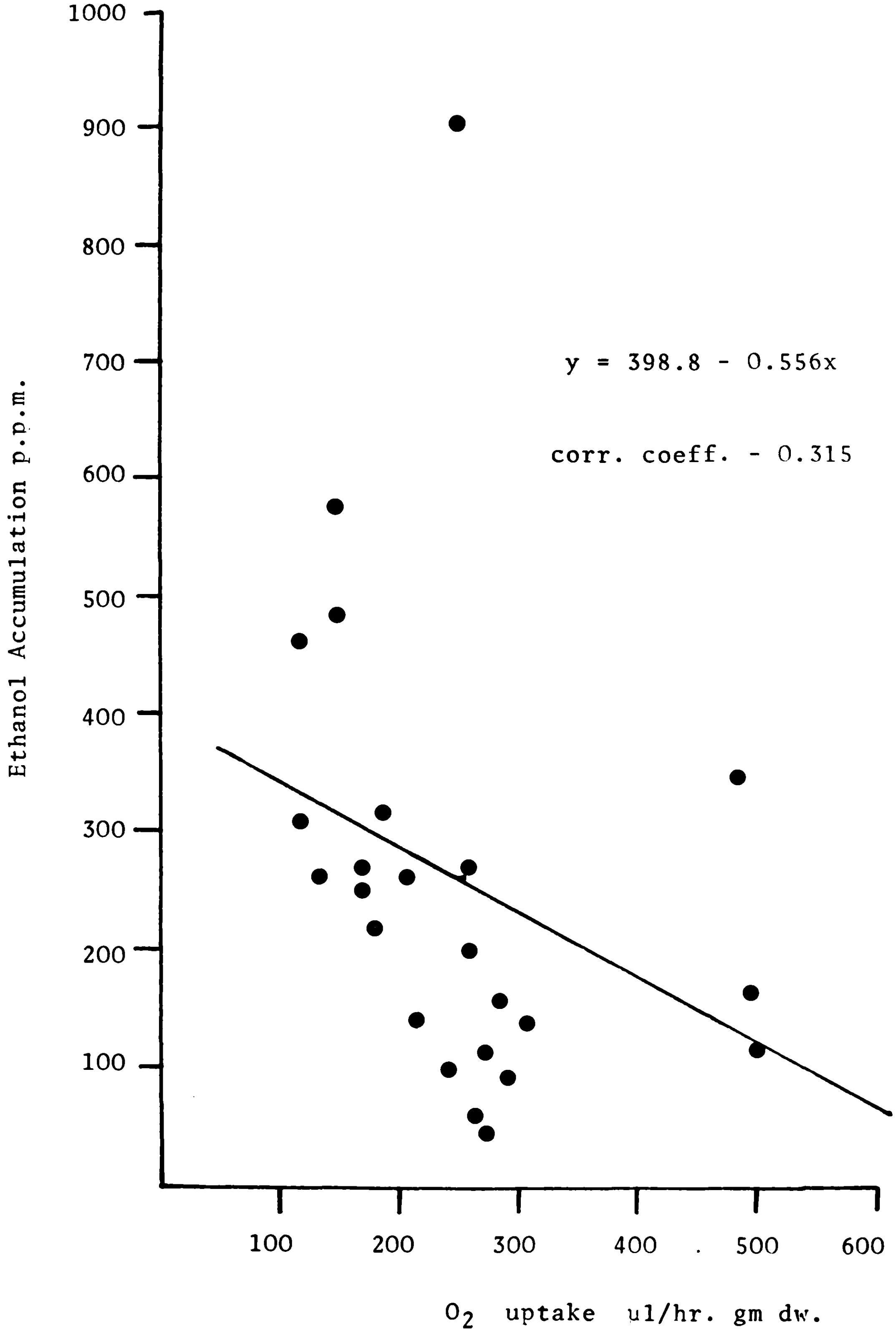

1000
900
800
700
600
500
400
300
200
100
Ethanol Accumulation p.p.m.
y = 398.8 - 0.556x
corr. coeff. - 0.315
100
200
300
400
500
600
O_2 uptake ul/hr. gm dw.

Fig. 19. The relationship between the level of ethanol accumulation and the rate of carbon dioxide evolution, of six seed lots of pea, after 24 hours in four substrate moistures at 20°C.

ETOH/CO_2

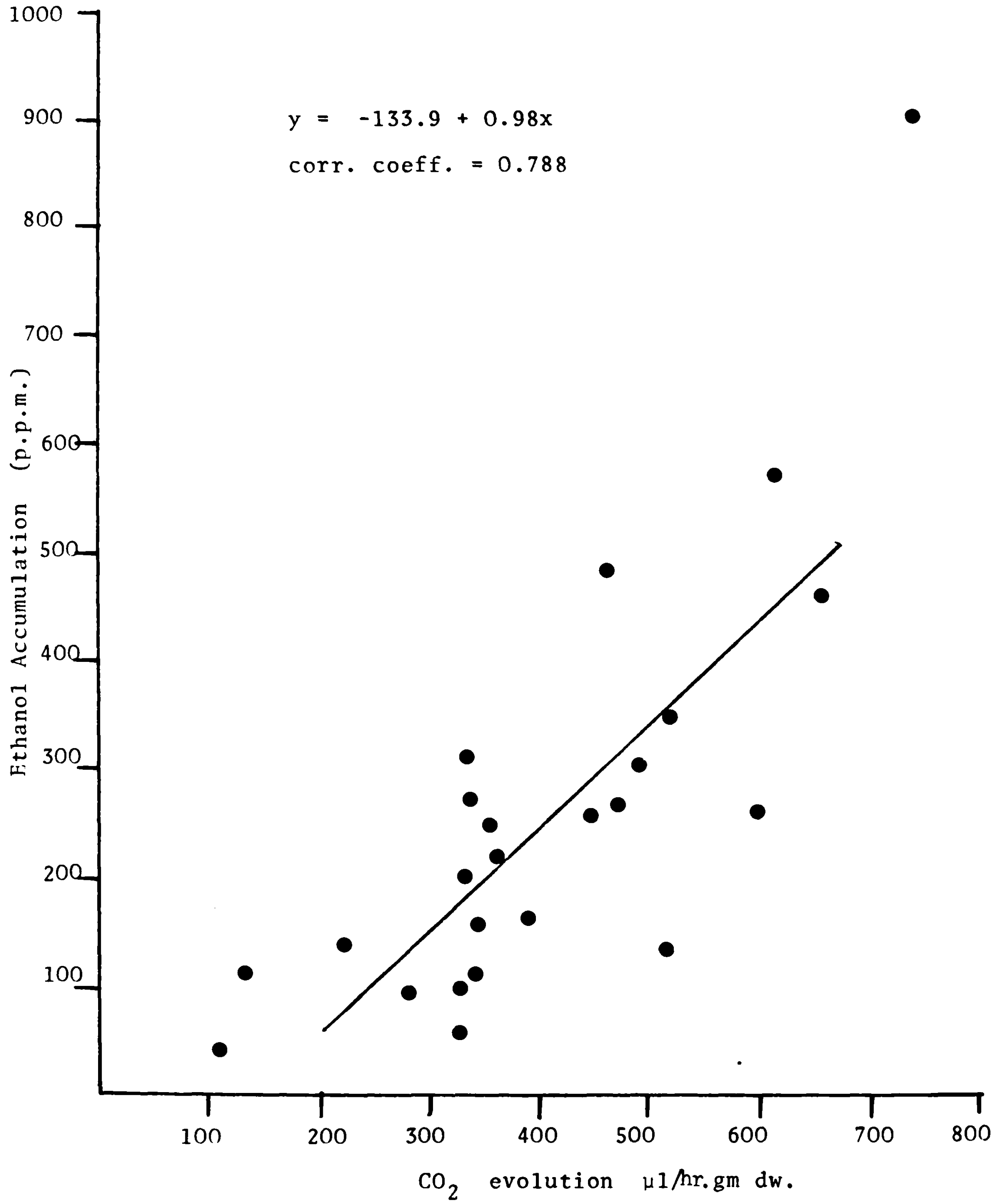

y = -133.9 + 0.98x
corr. coeff. = 0.788
1000
900
800
700
600
500
400
300
200
100
Ethanol Accumulation (p.p.m.)
100
200
300
400
500
600
700
800
CO2 evolution µl/hr.gm dw.

Fig. 20. The relationship between the level of ethanol accumulation and the respiratory quotient values of six seed lots of pea after 24 hours in four substrate moistures at 20°C.

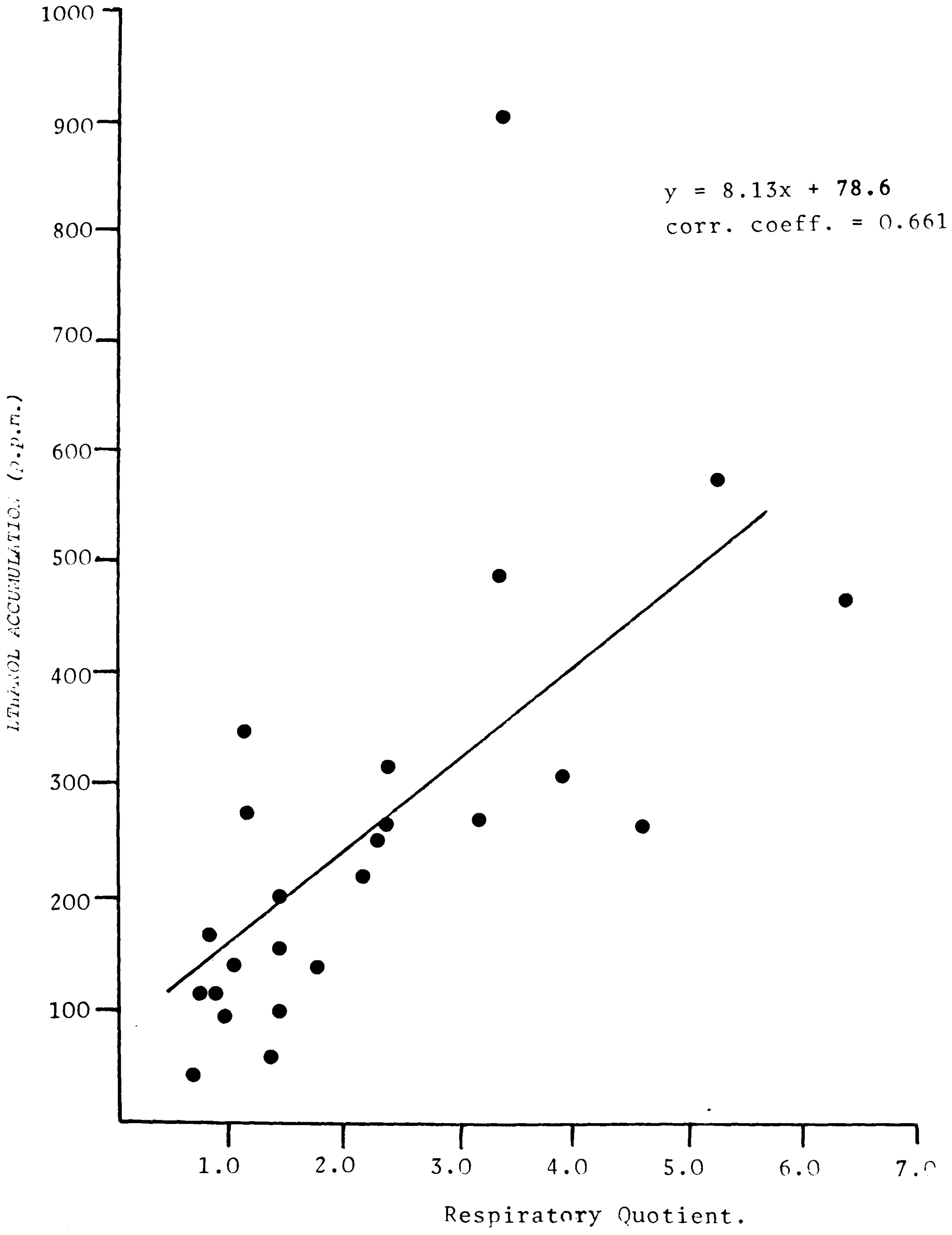

1000
900
800
700
600
500
400
300
200
100
ETHANOL ACCUMULATION (p.p.m.)
y = 8.13x + 78.6
corr. coeff. = 0.661
1.0
2.0
3.0
4.0
5.0
6.0
7.0
Respiratory Quotient.

Table 15. Mean Increase in CO_2 output, of five seed lots of pea, measured in nitrogen compared to their output in air.

Seed Lot.	% increase in CO_2 output in N_2 compared to air.
39	+ 70%
04	+ 29%
03	+ 34%
54	+ 49%
20	- 2%

Although the replicates shown in Appendix III showed considerable variation, the results do show that four of the five seed lots responded to anaerobic conditions with increased levels of carbon dioxide evolution. The good seed lot 39, had a very large increase in output under nitrogen, a 70% increase compared to the 2% decrease in nitrogen found in the poor seed lot. The increases in output of the intermediate seed lots were mid-way between those of the good, and the decrease of the poor seed lot, ranging from 29-49%. These responses are similar to those found in the seed lots, after they have been removed from the treatment columns. The anaerobic conditions of the treatment columns therefore influence the carbon dioxide evolutions of the seed lots in a similar manner to nitrogen gassing, indicating that the post-treatment measurements are indicative of the respiratory metabolism under those treatments.

(4) Exudation.

The exudation rates, expressed as the mean of six replicates of experiments conducted at 20°C, and of three replicates at 5^{o}C, are presented in Tables 16(a) and 16(b) and Fig. 21. The results are corrected for the basic conductivity of the sand.

Table 16. Mean exudation levels (μmho/Cm^{-2}/10 seeds) of the six seed lots subjected to the moisture and temperature treatments.

(a) 5^{o}C

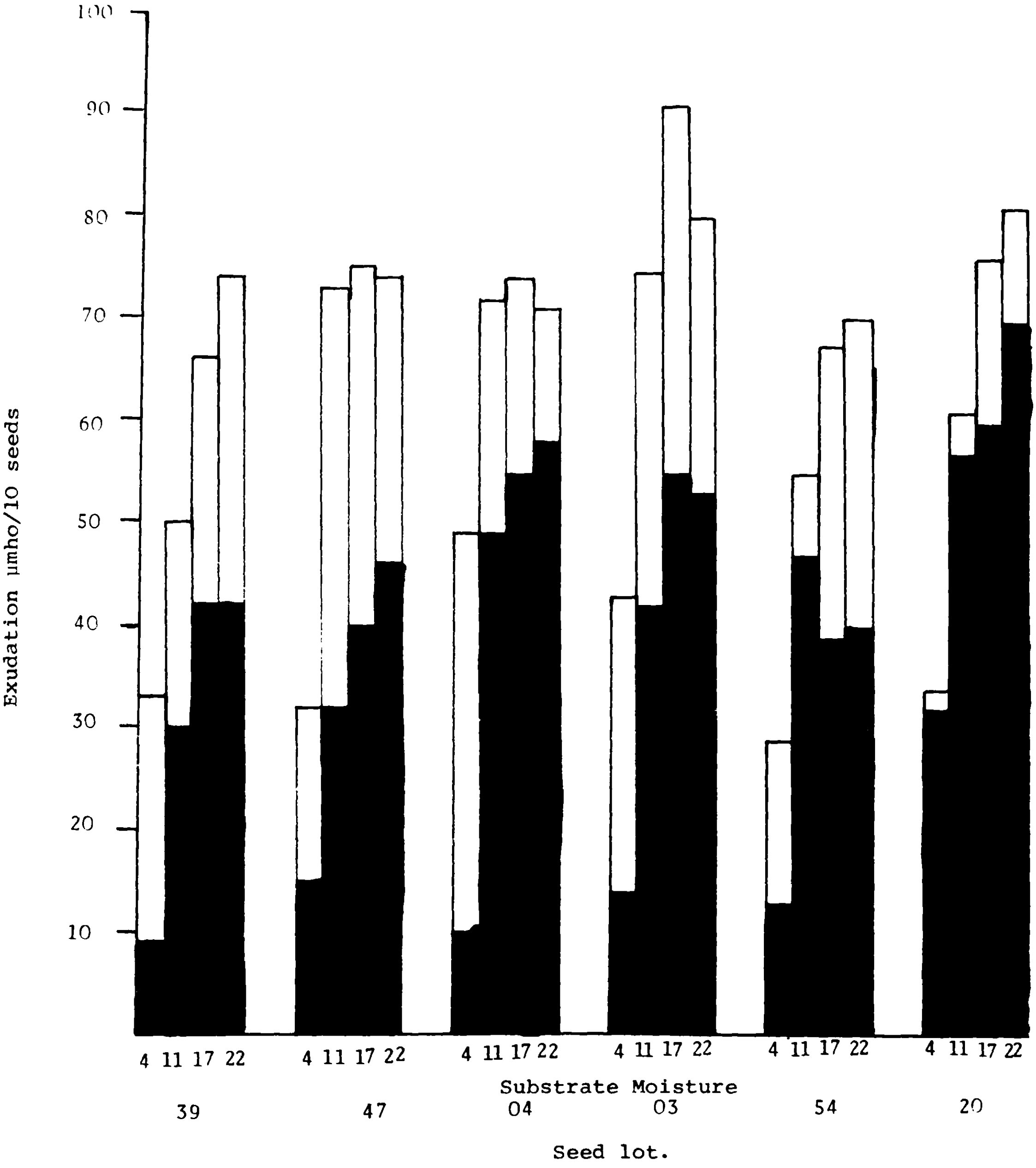
	Seed Lots					
Moisture	39	47	04	03	54	20
22%	74	74	71	80	70	81
17%	66	75	74	91	68	76
11%	50	73	72	75	55	61
4%	33	32	49	43	29	42

(b) 20^{o}C

	Seed Lots					
Moisture	39	47	04	03	54	20
22%	42	46	58	53	40	70
17%	42	40	55	53	39	60
11%	30	32	49	42	47	57
4%	9	15	10	14	13	34

The most noticeable feature was that the exudation rates of all the seed lots were higher at 5^{o}C than at 20^{o}C. At both temperatures the highest rates of exudation occurred at the highest substrate moistures, and the pattern of exudation related to substrate moisture was similar for all the seed lots. Exudation from the seeds increased as the substrate moisture increased. At the higher temperature the poor and intermediate seed lots exuded more than the good

Fig. 21. The levels of exudation (μhmo cm^{-2}) of six seed lots of peas whilst in four different substrate moistures for 24 hours at 5°C □, and 20°C ■; moistures are shown as percentage moistures of oven dry sand.


100
90
80
70
60
50
40
30
20
10
Exudation µmho/10 seeds
4 11 17 22
4 11 17 22
4 11 17 22
4 11 17 22
4 11 17 22
4 11 17 22
Substrate Moisture
39
47
04
03
54
20
Seed lot.

seed lots. This difference was less marked at 5°C, the noticeable feature at this temperature being the similarity in rates of exudation of all the seed lots, at the highest substrate moisture.

Table 17. Exudation of two intermediate seed lots in 6" columns, following treatments at four moisture contents.

04

Mean Exudation of 10 seeds (μmho) in the substrate moistures at 20°C.		Exudation at 11% moisture level after 24 hours in treatment columns.
22%	53	52
17%	52	68
11%	47	50
4%	20	43

54

Mean Exudation of 10 seeds (μmho) in the substrate moistures at 20°C.		Exudation at 11% moisture level after 24 hours in treatment columns.
22%	42	43
17%	38	36
11%	41	42
4%	15	37

When the seeds were replaced in 6" columns to measure their post-treatment rates of exudation, generally all the seeds had rates of exudation characteristic for 6" columns. The one major exception occurred in seeds from lot 04 that had previously been subjected to the 17% moisture level. They exhibited higher than expected levels of exudation when subsequently placed in an 11% moisture regime.

(5) Predisposition.

The fungus isolated from the cotyledon of the pea seeds was identified as Pythium ultimum. No embryo axis were seen to be infected in any of the seed lots, all fungal hyphae grew out of the cotyledons. However, isolations were only made after 48 hours in soil, and axis infection may have occurred after this time.

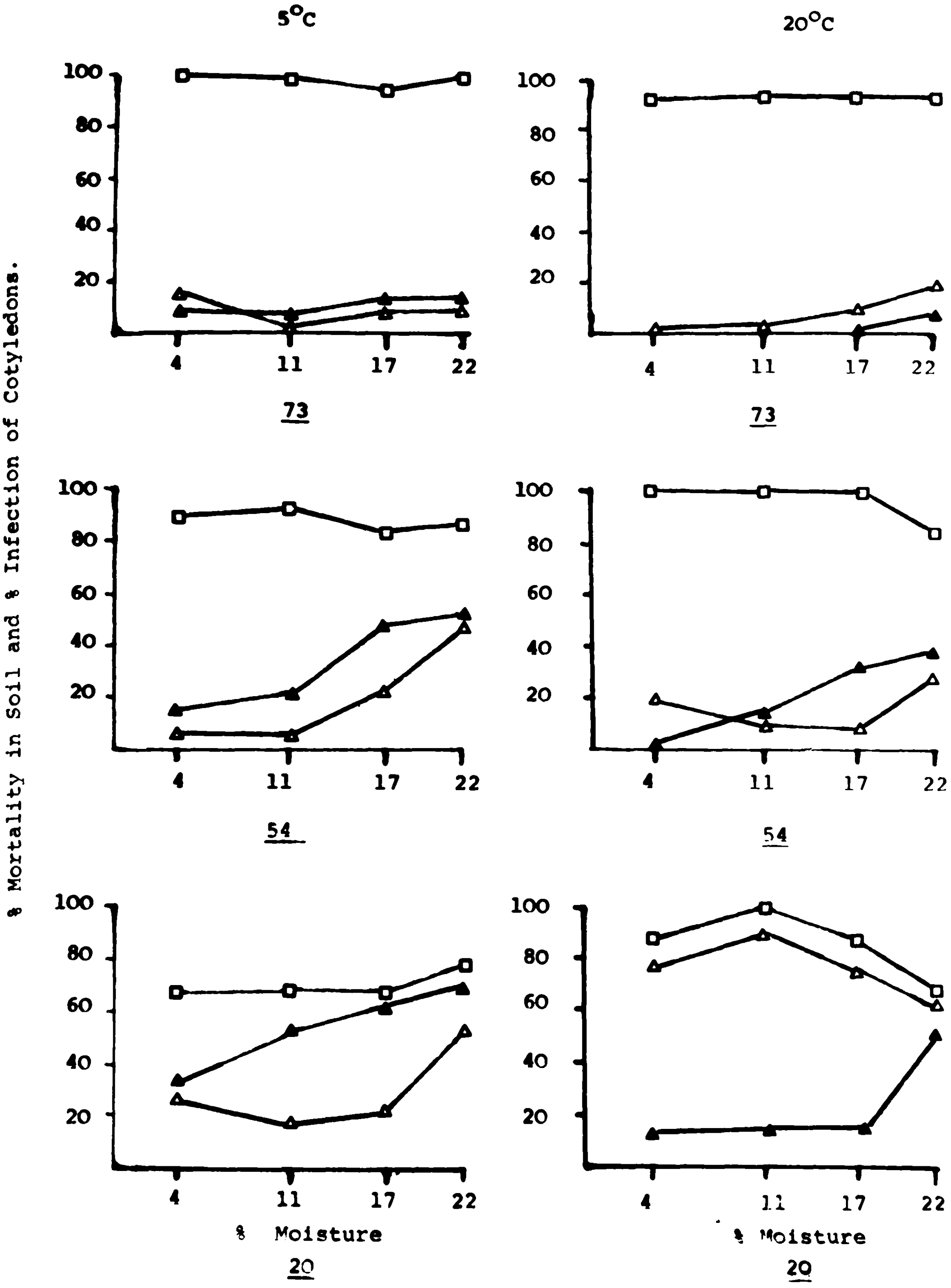
Table 18 shows the influence of the pretreatments on the viabilities of the seed lots, and also the mortality of the seed lots when sown in soil, corrected for any reduction in viability that the pretreatments may have caused.

Table 19 shows the extent of infection suffered by the seed lots after the various pretreatments, and all the results are presented graphically in Fig. 22.

After the 5°C pretreatments the good (73) and intermediate (54) seed lots had high levels of emergence in sterile sand indicating that the moisture pretreatments did not influence the viability of the seed lots. The poor seed lot (20) did suffer a reduction in viability, as many as 33% of the seeds failing to emerge, but surprisingly the largest reductions in viability occurred after the driest pretreatment.

When planted in soil the good seed lot showed very little pre-emergence mortality. However, the intermediate seed lot showed very heavy pre-emergence mortality in soil, particularly after the wettest pretreatment, the extent of mortality decreasing as the pretreatments the seeds obtained became drier. The pre-emergence mortality of the poor seed

Fig. 22. The influence of prior treatments of different substrate moistures and temperatures on the emergence of three seed lots of pea in sterile sand □; on the % mortality in unsterilized soil △ ; and on the % of cotyledon pieces with fungal infection, ▲ .

5°C
20°C
% Mortality in Soil and % Infection of Cotyledons.
100
80
60
40
20
4
11
17
22
73
54
20
% Moisture

lot was very high in soil, and also showed a slight increase when the seeds were given the wetter pretreatments.

The extent of fungal infection of the cotyledons of the three seed lots reflected the differences found in their mortalities in soil. The poor seed lot, and the intermediate seed lot that had received the 17% and 22% moisture pretreatments, showed heavy infection, but the good seed lot showed very little fungal infection after any of the pretreatments.

After pretreatments at 20°C, as opposed to 5°C, the good and intermediate seed lots showed less mortality in soil, but the poor seed lot showed more mortality. At 20°C there was even less difference between the emergence in sand and soil for the good seed lot, than at 5°C. The response of the poor seed lot after the pretreatments at 20°C, confirmed the findings at 5°C, that the seed lot was unable to attain a high level of germination in unsterilized conditions, irrespective of the pretreatment the seeds were given. In the intermediate seed lot, the same pattern of increased mortality in soil, and increased levels of fungal infection after the wetter pretreatments was found, agreeing with the 5°C findings, but mortality was not as high as at 5°C and the drops in infection and mortality accompanying each drier pretreatment were not as marked.

Table 18. % Emergence in Sand, and % Mortality in Soil (corrected for loss of viability due to pretreatments) of three seed lots subjected to the four moisture treatments at 5°C and 20°C.

	73 (good)				54 (intermediate)				20 (poor)			
	% Mortality in Soil		% Emergence in Sand		% Mortality in Soil		% Emergence in Sand		% Mortality in Soil		% Emergence in Sand	
	5°C	20°C	5°C	20°C	5°C	20°C	5°C	20°C	5°C	20°C	5°C	20°C
4%	14	3	100	93	5	20	88	100	26	78	67	88
11%	2	2	100	93	5	10	93	100	17	90	67	100
17%	9	9	95	93	21	5	83	100	21	77	67	88
22%	11	20	100	93	49	26	88	83	53	62	77	67

Table 19. % Fungal infection of Cotyledon pieces from soil after seed pretreatments of four moisture treatments at 5°C and 20°C.

Percentage of cotyledon pieces with infection.

Moisture Pretreatment	73 (good)		54 (intermediate)		20 (poor)	
	5°C	20°C	5°C	20°C	5°C	20°C
4%	9	0	15	0	33	12
11%	6	0	20	12	54	12
17%	12	0	48	30	61	16
22%	12	6	52	36	68	50

DISCUSSION

The respiration rates of seeds during the early hours of germination are related to the water contents of the seeds (Opik & Simon, 1963). Furthermore, the respiration rates of seeds, being metabolic processes are influenced by temperature. Both Mayer and Poljakoff-Mayber(1963) and Spragg and Yemm (1959) have commented, however, that the increases in respiratory rates of pea seeds during imbibition were not as high as expected, when the temperature was increased. This was probably due to the testas of the seeds limiting gaseous diffusion, until they were penetrated by the radicles. By pretreating the seeds used in these experiments to one moisture content, and by measuring the influence of the different treatments after 48 hours, before radicle emergence, both the problems of seed moisture content, and condition of the testa were overcome. Any differences that existed between the seed lots were attributable to the different moisture and temperature treatments the seed lots received.

The respiration rates of the seed lots were measured in air, after the different sand column moisture treatments, and they were regarded as indicative of the respiration rates the seed lots used whilst subjected to the different treatments. Comparisons of respiration under nitrogen, with respiration after the treatments showed that the carbon dioxide outputs of the seed lots, measured in air, after a period of anoxia, were similar to the outputs during a period of anoxia. The post-treatment measurements could therefore be regarded as indicative of the respiration rates of the seed lots during the treatments.

The one good seed lot tested responded to anaerobic conditions with a large carbon dioxide output, 70% higher than in air, similar to the increase shown by the good seed lot when it was subjected to the wettest moisture treatment. The poor seed lot, however, both after the wettest moisture treatment and during the experimental anaerobic period, showed a slight decrease in carbon dioxide output.

All the seed lots used in these experiments exhibited lower oxygen uptake and carbon dioxide evolution values at 5°C than 20°C. Reduction in the respiratory activity of seeds as a result of low temperature, have been noted previously for beans (Opik and Simon, 1963, Orphanos and Heydecker, 1968), lima beans (Woodstock and Pollock, 1965), Birds Foot Trefoil (Qualls and Cooper, 1968), and cotton (Hayman, 1969) but there is no evidence available demonstrating the influence of low temperature on the respiratory activity of individual seed lots within one cultivar. These results have shown that the differences that existed between the seed lots at 20°C were retained at 5°C, the only effect of low temperature being an overall reduction in the respiration rates of the seed lots Low temperatures, therefore, did not accentuate or reduce the basic differences in respiratory activity that existed between the individual seed lots.

However, these basic metabolic differences between seed lots are strongly influenced by the different substrate moistures. Five of the six seed lots produced more carbon dioxide at the wettest substrate level than the driest one, a response that Sherwin and Simon (1969) have shown occurred in *Phaseolus* spp. seeds subjected to high moisture conditions. The only seed lot that did not show this increase in carbon dioxide output was the seed lot known to suffer from a high level of pre-emergence mortality in most soil conditions.

This seed lot also differed from the others in the response of its oxygen uptake to the increased substrate moistures. The poor seed lot showed successive drops in oxygen uptake, as the moisture level increased, whereas the good seed lots were able to maintain a high oxygen uptake until they were subjected to the wettest substrate moisture. This inability of the poor seed lot, and to a lesser extent the intermediate seed lots, to maintain high levels of oxygen uptake under wet conditions, may play an important part in the failure of these seed lots to emerge in wet soils.

A reduction in oxygen uptake during germination may mean a seed's energy requirements are not met unless it is capable of a high rate of anaerobic respiration. The R.Q. values calculated for the poor seed lot illustrated that it was incapable of producing large increases in its level of anaerobic respiration as the substrate moisture increased. The R.Q. values of the good seed lots indicated that they were able to maintain aerobic pathways, until they were subjected to the wettest treatments, when they adopted a very high level of anaerobic respiration.

Low temperatures and high substrate moistures influenced the respiratory activity of seeds, but perhaps more importantly to a study of establishment, individual seed lots within one cultivar were influenced to varying degrees.

The ethanol accumulation data also indicated that the rates of metabolism of the seed lots were influenced by the different temperature and moisture treatments. The reduction in metabolic activity at 5°C compared with 20°C led to lower levels of ethanol accumulation in all the seed lots, but temperature did not influence the basic differences in accumulation rate that existed between the seed lots. However, the different substrate moistures caused some marked differences in response by the seed lots.

The accumulation of ethanol under anaerobic conditions is a common feature of higher plants (Vishniac, 1957), and increased levels of ethanol accumulation under conditions of high substrate moisture have been demonstrated in the roots of sugar beet (Stiles and Dent, 1947; James, 1953; Kenefick, 1962), maize and sunflowers (Grineva, 1963), the seeds of *Phaseolus* spp. (Sherwin and Simon, 1969) and the xylem sap of tomatoes (Fulton and Erickson, 1964, Bolton and Erickson, 1970).

Crawford (1966, 1967) working largely with species of *Senecio*, differing in their tolerance to flooding, suggested two ways in which plants could withstand oxygen deficiencies. Firstly, they can develop a means of controlling the production of the end products of anaerobic respiration, or secondly, they can be tolerant of high levels of those end products. His work demonstrated that certain species of *Senecio* were capable of controlling the production of the end products of anaerobic respiration.

Plants tolerant of flooding produced two or three times less ethanol than plants intolerant of flooding, when their roots were flooded. A high level of alcohol dehydrogenase activity (ADH) was also noted in intolerant plant roots grown in low oxygen conditions. Acetaldehyde, resulting from the decarboxylation of pyruvate during conditions of restricted oxygen supply, has been shown by Kollofel (1968) and Hageman and Flesher (1960) to induce alcohol dehydrogenase activity. Crawford and McMannon (1968)noted that acetaldehyde induced ADH activity more in the intolerant than tolerant plants.

Crawford (1969) also showed that in conditions of low oxygen availability, some plants tolerant of flooding switched from ethanol to malic acid accumulation. The avoidance of the accumulation of ethanol under conditions of low oxygen has also been reported in the rhizomes of *Iris pseudoacornus* (Boulter, Coult and Henshaw, 1963), where besides malic acid, shikimic acid was also accumulated.

An example of the second method by which plants can withstand high moisture conditions, that of being able to tolerate high concentrations of the end products of anaerobic respiration, is found in the work of Hook, Brown and Kormank (1971). They found that roots of Swamp Tupelo (*Nyssa*) tolerated flooding by an increased rate of glycolysis, a reaction that Crawford found in *Senecio* spp.intolerant of flooding, and that the roots accumulated high concentrations of ethanol. Instead of avoiding the accumulation of ethanol, they were capable of tolerating it, in high concentrations. An increase in anaerobic respiration, similar to that demonstrated in the roots of *Nyssa*, has been shown by Taylor (1942) to be the mechanism which enables rice seeds to germinate in low oxygen concentrations.

The experimental treatments of high substrate moisture, used in these experiments, lasted only 24 hours, a situation likely in agricultural soils since long flooding periods are unusual. Nevertheless, it is useful to compare the ethanol accumulation rates of the seed lots, with those of the plants mentioned above, bearing in mind that some of the experimental flooding periods used in the work reported, lasted up to one month.

It is apparent from the levels of ethanol accumulated by the seed lots subjected to the highest moisture treatments, that none of the seed lots were capable of avoiding the accumulation of ethanol during anaerobic conditions. The good seed lots exhibited the same pattern of ethanol accumulation as described by Hook et al, for plants capable of tolerating flooding. The ethanol productions, when the seed lots were subjected to the wettest treatments, were twelve times larger than those in the driest treatments at 20°C and seven times as large as that in the driest treatment at 5°C. The smaller amounts of ethanol accumulated at the lower temperature were due to the overall slowing down of the metabolic processes, a result also noted by Bolton and Erickson (1970) studying tomato sap.

Besides influencing their physiology, the treatments of low temperature and high moisture also influenced the soil emergence of the seed lots. The intermediate seed lot in particular, suffered heavy pre-emergence mortality in soil after being subjected to the wettest substrate moisture treatment. This mortality may have arisen from the treatments influencing the viability of the seeds or their predisposition to fungal attack.

Low temperatures have long been recognised as the cause of low seed establishment in some crop seeds by reducing the viability of the seeds. The phenomenon of chilling injury, demonstrated in cotton (Christiansen, 1968,1969) and lima bean seeds (Pollock and Toole, 1966; Pollock, 1969) imbibed at low temperature was suggested as the cause of emergence failures of these two crops after low temperatures in the field. Chilling during germination has also been shown to cause reduced survival in crimson clover (Hoveland and Elkins, 1965), cacao (Ibaney, 1963), peas (Highkin and Lang, 1966) and garden beans (Pollock, Roos and Manalo, 1969) and Pollock (1969) suggested the phenomena to be of general significance to plant establishment, because of the range of plants involved.

The mechanism of chilling injury was suggested by Woodstock and Pollock (1965) to be a purely physical injury to the seeds. During low temperature imbibition the respiratory mechanism is too slow to supply sufficient energy for orderly stretching of the cell membranes. Extensive damage may occur, an indication of this being the release of organic materials from seeds affected by chilling injury. Later theories suggested that death of seeds through chilling injury, was the result of a block in a metabolic system (Christiansen, 1968) or a combination of physical and metabolic injury to the seeds (Pollock, 1969; Obendorf and Hobbs, 1970)

The ability of the seeds to withstand the initial inrush of water when they are placed in soils of high moisture content has also been suggested as an important factor which may influence the viability of the seeds. Eyster (1940) found that germinating seeds wee not killed by soaking, if they were first allowed to imbibe slowly, and later Larson (1968) found that pea seeds imbibed without their testes suffered more injury than intact seeds. This also indicated that if imbibition took place slowly, germination would be successful. It has been suggested that this sudden inrush of water does not enable the seeds to achieve the level of subcellular organisation necessary for successful germination.

A third way in which the treatments may reduce the viability of the seed lots is apparent from the work of Crawford (1969). He suggested that non-helophytes may eventually die from the accumulation of toxic quantities of ethanol. This may apply to the seed lots that failed to emerge after being subjected to the wettest substrate moisture. However, as the seed lots that had the highest rates of ethanol accumulation after the wettest moisture treatments, retained their high levels of viability, this explanation does not seed to hold in the case of these seed lots. The results reported here indicate that a reduction in viability, caused by the treatments, could not account for the differences in soil emergence of the seed lots. After the short exposure to the different treatments, only one seed lot exhibited any purely physiological death, and then it caused only a 33% reduction in emergence. If the treatments did not affect the viabilities of the seed lots to any great extent, then differences in field emergence must have arisen from the treatments influencing the seeds' predisposition to pre-emergence mortality.

Two ways in which the treatments may influence the seeds' predisposition to pathogenicattack have been suggested. Firstly, the treatments may influence the exudation rates of the different seed lots, to the extent that some seed lots suffer heavy pathogenicattack. Secondly, recent work by Matthews (1971) indicated that important differences between seed lots lay in the readiness with which their cotyledons were infected, and it may be that some of the treatments influence the predisposition of the cotyledons to pathogenic attack.

The exudation rates of the seed lots were markedly influenced by substrate temperature and moisture. The exudation rates were greater at 5°C than 20°C, and increased with increasing substrate moisture. Increases in seed exudation as a result of low temperatures have been noted for mung beans (Kraft and Erwin, 1967), cotton (Hayman, 1969) and peas (Perry and Harrison, 1970). In contrast, Vancura (1967) reported that the exudation rates of maize and cucumber seeds increased as temperature increased. Unfortunately, the seeds used in his experiments were not of the same physiological age, a problem overcome in these experiments by pretreating all the seeds. Low temperatures also increase the exudation rates of plant roots, more amino acids being released from strawberry roots at 5-10°C than at 20-30°C, and Husain and McLean (1963) suggested that this could be the cause of the increased attack by <u>Rhizoctonia fragariae</u> at low temperatures.

High pre-emergence mortality of pea seeds is associated with high soil moistures as well as low temperatures, and in these investigations, seed exudation increased as a result of increased substrate moistures. Increases in seed exudation as a result of high substrate moistures were recorded by Kerr (1964) using an indirect measure of exudation. He expressed exudation by measuring the decrease in dry weight of seeds, and although he pointed out that respiration did influence the results, he did recommend that a more direct measure of exudation would be more satisfactory. The method used in these studies, that of washing the sand in which the peas had been treated, and measuring the conductivity of the wash-water using a conductivity meter (Matthews and Bradnock, 1968) is certainly a more direct method of measuring exudation.

Although temperature influenced the exudation rates of the seed lots in a uniform way, the seed lots did not respond in a uniform way to the increases in substrate moisture. Seed lots that exhibited high pre-emergence mortality in the field, rapidly attained a high level of exudation as substrate moisture increased. These increases in exudation were not as apparent in seed lots that exhibited low pre-emergence mortality in the field.

Initially, therefore a relationship between pre-emergence mortality and a high level of exudation under high substrate moisture conditions seemed to exist. However, when all the seed lots were subjected to the wettest substrate moisture, they exhibited very similar levels of exudation.

Although this study has demonstrated that low temperatures and high substrate moistures increased the rates of exudation of pea seeds, it has also confirmed the suggestions of Flentje and Saksena (1964) and Cook and Snyder (1965) that these levels of exudation cannot be the only factor responsible for differences in pre-emergence mortality of pea seeds. Besides having similar levels of exudation at the highest substrate moisture, the post-treatment determinations of exudation indicated that when marked differences in emergence occurred between the seed lots, following different moisture treatments, there were little differences in exudation.

The response of the seed lots to different soil conditions does not seem to be due only to differences in exudation between the seed lots. The important influence of soil conditions may be their effect on the predisposition of seeds to soil infection. Soil conditions may influence the physiological condition of seeds, not to the extent of causing a reduction in viability, but enough to predispose some seed lots to pathogenic attack. Schulz and Bateman (1969) suggested that the reduction in respiration caused by low temperatures may lead to increased susceptibility of seeds to pathogenic attack, by hindering the host from eliciting its natural resistance mechanisms. As this work has also shown that increasing substrate moistures reduces the respiration rates of seeds, moisture may also have a similar effect.

The physiological condition of the individual seed lots, as represented by the respiratory and ethanol accumulation data, differed greatly when they were subjected to similar conditions of temperature and moisture. Under conditions of high moisture the good seed lots adopted vigorous levels of anaerobic respiration, but the poor seed lot did not increase its rate of respiration from the level it adopted during the driest treatment.

From the evidence presented above, the following hypothesis is advanced to account for the differences in pre-emergence mortality that occurred between the seed lots, subjected to the same adverse conditions. During conditions of high moisture and low temperature the exudation rates of the seed lots increased, and stimulated greater pathogenic activity in the soil surrounding the seeds. Some seed lots, even though they had high levels of exudation, may however have been in a physiological condition that enabled them to withstand pathogenic attack. They may have retained aerobic pathways of respiration, or in very anaerobic conditions, adopted anaerobic pathways so that the seeds remained in a physiological condition which enabled them to withstand pathogenic attack by Pythium spp.

In contrast, seeds from poor seed lots were unable to increase their levels of respiration and the combination of high seed exudation, and poor physiological condition, predisposed the seeds to pathogenic attack.

Emergence failures during adverse conditions are therefore suggested to be due to the additive effect of seed exudation, and the physiological condition of the seeds, predisposing them to infection. In this investigation, only rarely did the failures result from the conditions causing purely physiological death of the seeds.

SECTION IV

A PREDICTIVE EQUATION FOR FIELD EMERGENCE

INTRODUCTION.

Mathematical expressions of the relationship between seed conditions and field emergence have been produced for castor beans (Thomas, 1960) and peas (Bradnock & Matthews, 1970). In both instances conductivity measurements of seed-steep water were used to describe seed condition.

Soil conditions, notably moisture and temperature, have been shown to have a marked influence on emergence (Harper, Landragin and Ludwig, 1955; Perry, 1970). Furthermore, the work described in Section II has shown that soil conditions adversely affect some lots much more than others. Thus, mathematical expressions which include only conductivity and seed weight (Bradnock & Matthews, 1970), have limited value, especially for seed lots of the intermediate category, whose exudation is that of a good seed lot, but whose emergence is low under low temperature and high moisture soil conditions and improves as the soil gets warmer and drier.

The availability of computers has led to a greater use of multiple regression techniques which enable the incorporation of many variables into regression equations, and have been used in the past to produce predictive equations for field emergence of peas (Bradnock & Matthews, 1970).

Multiple regression techniques have been used in a wide range of biological situations. For instance, Searle (1970) used these techniques to produce a predictive equation to estimate the body weight of sheep from several simple parameters, and Morris (1959, 1963a, 1963b) claimed that it was possible to interpret population changes of the spruce budworm, from one generation to the next when only one or two mortality factors were measured.

When faced with many variables, for example environmental parameters, all of which could influence the outcome of some event, such as field emergence, multiple regression techniques are useful in two ways. Firstly, they can assist in detecting the most important parameters, by measuring the amount that the variability is reduced by the successive elimination of a large number of parameters in turn, and secondly, they can produce a predictive equation that may be useful in future situations.

Heydecker (1968) suggested that an absolute prediction of field performance could never be achieved, but that it may be possible to obtain a ranking of seed lots, better than that obtained from the conventional germination test. With this point in mind, therefore, an attempt was made to improve the existing predictive equations for seed emergence, by introducing components representing the weather conditions, at, and shortly after, the time of planting.

MATERIALS & METHODS

The initial computation was conducted on the emergence data, corrected for the differing viabilities of the lots, for the seed lots 39, 47, 04, 03, 54, 80, 21 and 20, sown at the Field site, in the emergence experiment described in Section II. The meteorological data collected during the duration of the experiment, was used to produce the weather variables related to the emergences of the seed lots, at the different planting dates.

'Moving means' obtained by taking the mean values of weather variables for differing periods before and after the date of planting, were calculated for each weather variable. These 'moving means' ranged for example, from the mean maximum 2 in (5 cm) temperature three days either side of planting, to the mean maximum 2 in (5 cm) temperature two weeks after planting. Several means were obtained for each particular meteorological measurement, and these were correlated against the emergence of the seed lots, at all the planting dates. This procedure enabled the 'moving mean' for each measurement, that was most closely associated with the emergence of the seed lots, to be singled out.

The technique of multiple regression was then employed, to fit a linear relationship between percentage emergence (y) and the suspected influencing variables x, x_2 ... x_n, of the form:-

$$y = a_0 + a_1 x_1 + a_2 x_2 \dots a_n x_n \qquad (1)$$

where the a_i, $i = 0,1, \dots n$, are the regressions constants to be determined. Selective multiple regression was then used in an attempt to reduce the number of variables in equation (1), and to form a new equation:-

$$y = b_0 + b_1 x_1 + b_2 x_2 \dots b_m x_m \qquad (2)$$

with the number of variables, m, now included, being less than n, in such a way that only those variables of importance in their influence on y (emergence) were included.

The accuracy of any equation produced could not be tested using the data that was used to formulate the equations. A second field experiment was conducted, therefore, to produce emergence data to test any predictive equations produced by these techniques.

This second field experiment, conducted in 1971, used six seed lots of Dark Skinned Perfection, obtained from Charles Sharpe & Company. Laboratory determinations of exudation were by the method described in Section I. Replicated field plantings of these six seed lots, similar to those in Section II, were made on two occasions in 1971, 16th March and 23rd March, at the Field site. The emergence of the lots were recorded, and meteorological data were collected during the course of the plantings.

RESULTS

The mean 1970 emergence figures for the eight seed lots at the eight planting dates, are shown in Table 20, after arc sin transformation. The individual results of the four replicates, at each planting date, are presented in Appendix IV. The conductivity results, obtained from the bulk test, and expressed in μmho cm^{-2}, are also shown in Table 20.

Table 20. The exudation levels of the eight seed lots, and their arc sin transformed mean emergences at the eight planting dates.

Seed Lot	Planting Date								Exudation (μmho cm^{-2})
	1	2	3	4	5	6	7	8	
39	69.1	74.6	74.8	76.7	80.2	72.7	80.7	74.3	158
47	58.1	67.3	69.8	75.3	77.8	70.4	73.7	76.0	207
04	27.3	38.4	46.5	67.3	54.4	56.5	66.2	57.8	278
03	50.8	58.7	65.3	71.1	77.3	74.9	76.6	69.3	197
54	34.9	52.7	57.5	70.3	72.5	69.8	72.0	65.0	190
80	3.6	2.8	4.8	21.3	24.8	27.5	36.5	18.9	480
21	2.8	1.0	12.3	26.3	25.6	28.9	39.8	20.2	475
20	11.7	15.6	23.2	33.7	36.9	35.6	46.2	29.2	490

The first analysis considered the relationship between percentage emergence, y, and the exudation of the seed lots, omitting two seed lots 04 and 80, so that some data was left to investigate the validity of any relationship that may be evolved. The two seed lots were chosen before any of the analyses were conducted.

When the percentage emergence of the six remaining lots is plotted against log e, exudation, x, a linear relationship holds (Fig. 23). The regression line shown in Fig. 23 was fitted to the individual replicate emergences, and not to the seed lot means. The actual equation was:-

$$y = 311.213 - 46.3437x \qquad (3)$$

and was very significant, with $P < 0.001$. Seed lots 04 and 80 are also shown in Fig. 23 to verify the usefulness of equation (3).

Simply using the conductivity figures obtained for the seed lots gives a reliable indication of their field emergences. However, in Fig. 23, the range of percentage emergence values at each planting date are given. It is these residuals or deviations from the regression line that it is hoped the use of weather variables will explain, and in doing so, provide an even more reliable indication of potential field emergence.

Table 21 illustrates the correlation coefficients obtained relating different 'moving means' for each weather variable to the percentage field emergence of the eight seed lots. The significance of the correlation coefficients are indicated, and the 'moving mean' for each variable, selected as the one most related to the emergence of the seed lots, is underlined.

Fig. 23. The regression of percentage field emergence at the field site on the log e (exudation) of the seed lots of peas. The vertical lines indicate the range of emergence of the four replicates of each seed lot.

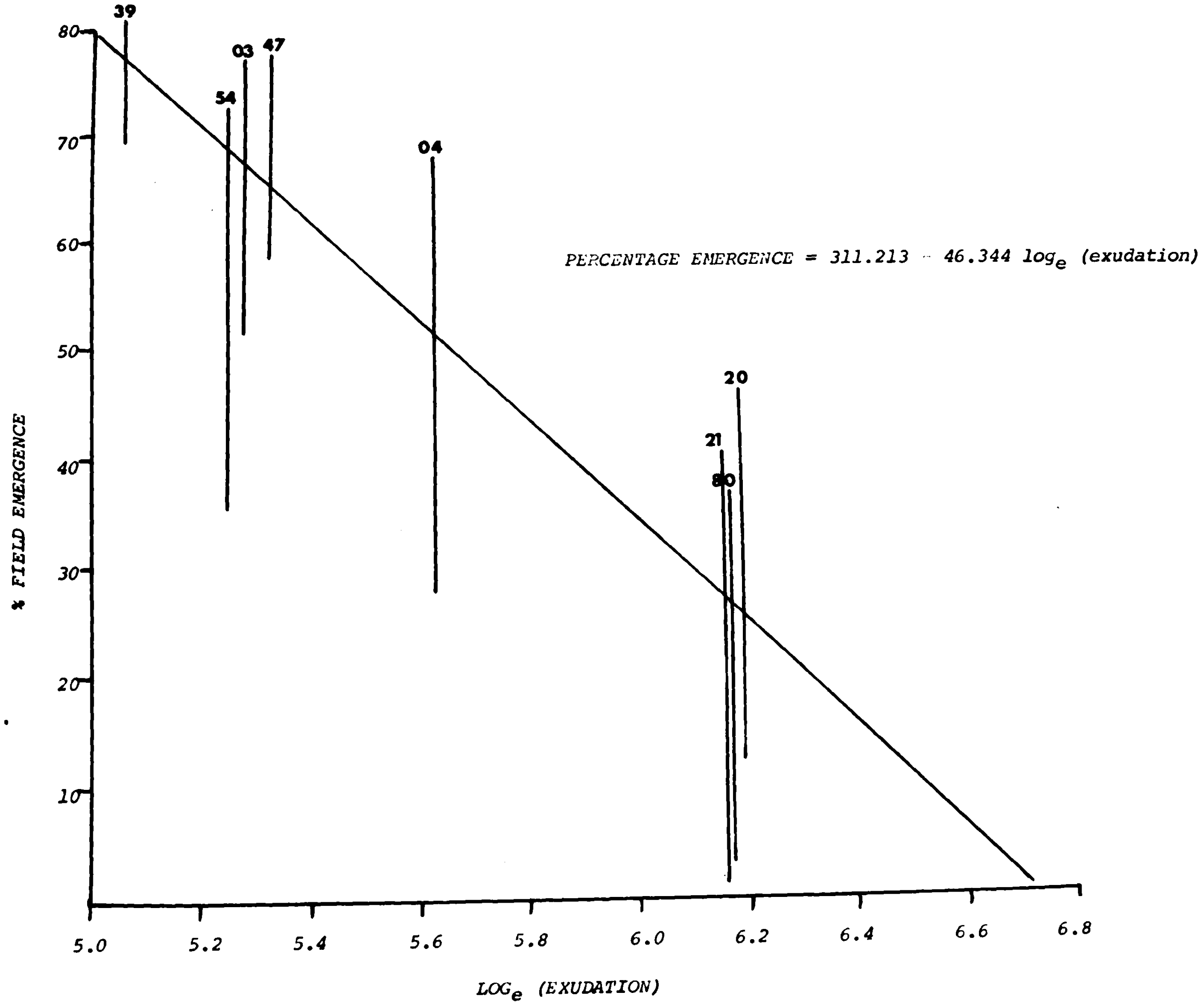

PERCENTAGE EMERGENCE = 311.213 − 46.344 $\log_e$ (exudation)
% FIELD EMERGENCE
80
70
60
50
40
30
20
10
LOG_e (EXUDATION)
5.0
5.2
5.4
5.6
5.8
6.0
6.2
6.4
6.6
6.8
39
54
03
47
04
21
80
20

Table 21. Correlation coefficients between different 'moving means' for each weather variable, and the emergence of the eight seed lots.

Source of 'moving mean'	1 ft soil Temp (^{O}F)	Air Temp (^{O}F)	2 in soil Temp (^{O}F)	4 in soil Temp (^{O}F)	Rainfall (mm)	Hours Below $50^{O}F$
Mean Maximum Temp. 3 days before and 3 days after planting 3/3 max.	0.354*	0.365*	0.377*	0.369*	0.048	n.c.
1/5 max.	n.c.	0.373*	0.383*	0.376*	0.066	n.c.
3/3 min.	n.c.	0.369*	0.369*	0.361*	-0.095	n.c.
1/5 min.	n.c.	0.353*	0.366*	0.371*	0.070	n.c.
1 week after min.	n.c.	0.346*	0.374*	0.354*	0.120	0.365*
2 weeks after min.	n.c.	0.360*	0.382*	0.383*	0.110	0.378*

(* = P < 0.05, n.c. = not calculated).

The weather variables selected are given in Table 22, the individual values for each planting date are shown in Appendix V.

Table 22. The minimum, mean, and maximum values recorded for the six weather variables, used in the calculations.

Weather Variable			Min.	Mean	Max.
1 ft Temp.	°F	W_1	38.3	49.6	61.5
Air Temp	°F	W_2	45	55.7	68
2 in Soil Temp.	°F	W_3	44	61.3	82
4 in Soil Temp.	°F	W_4	36	47.5	57
Rainfall (mm)		W_5	0.2	10.9	27.2
Hours below 50°F			0	110	335
log e (above + 1)		W_6	0	3.4	5.8

To include the weather variables in the predictive equation, a new variable, z, was introduced, so that:-

$$z = y - 311.213 + 46.3437x \qquad (4)$$

and then an attempt was made to relate z to the weather variables by use of the linear relationship:-

$$z = C_0 + C_1 W_1 + C_2 W_2 \ldots . C_6 W_6 \qquad (5)$$

As equation (5) was a linear model it was necessary to linearise the weather data, before analysis, where appropriate. The variable W_6 was fourmed, therefore, by the logarithm of hours below 50°F after adding unit to the data to avoid the logarithm of zero.

The selection procedures were then applied to (5). They suggested that the weather variable of primary importance was 4 in (10 cm) soil temperature, W_4, followed by hours below 50°F W_6, then 2 in (5 cm) soil temperature W_3, air temperature W_2, followed by rainfall W_5 and 1 foot temperature, W_1.

A significant fit was obtained ($P < 0.001$) by using W_4 and W_6 alone, the equation being:-

$$z = -97.40 + 1.878\ W_4 + 2.418\ W_6 \qquad (6)$$

and there seemed little to be gained by adding further variables to this equation.

In Fig. 24, the observed percentages emergences are compared with the expected emergences, obtained from the equation combining (4) and (6):-

$$y = 213.813 - 46.3437x + 1.878\ W_4 + 2.418\ W_6 \qquad (7)$$

for seed lots 04 and 80, at the eight different planting dates. The range of individual replicate values is shown for the two seed lots, the values presented being the arc sin transformed percentage emergences.

For the seed lot 04, the predictive equation (7) gives a reasonably satisfactory description of the percentage emergence over the whole season. For seed lot 80, however, the predicted percentage is nearly always in excess of those actually realised, especially in the earlier part of the season.

If the weather variables had been ignored and equation (3) used as the predictor, then the expected transformed emergences throughout the season for lots 04 and 80 would have been 50.2 and 25.1 respectively. The use of the equation containing the weather variables is therefore more accurate than the equation based on exudation alone.

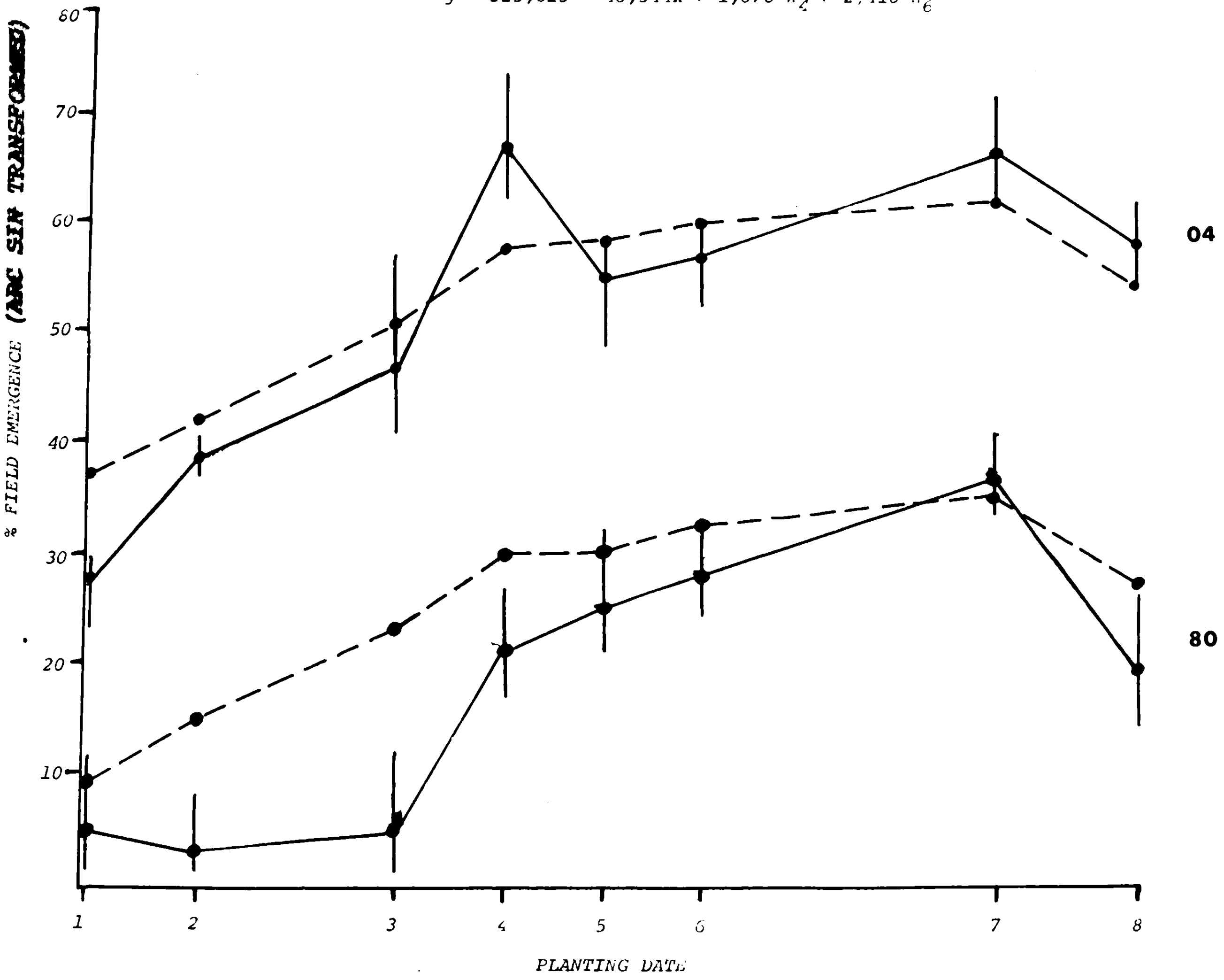
Fig. 24. The relationship between the observed emergence at the field site ●——; for two seed lots of peas, and their expected emergences values, ●- - -, calculated from the equation:

$$y = 213.813 - 46.3437x + 1.878\ W_4 + 2.418\ W_6$$

The vertical lines represent the range of field emergences of the four replicates of the seed lots, at the eight planting dates, at the field site.

VERIFICATION OF THE VALIDITY OF THE PREDICTION EQUATION

$$y = 213{,}813 - 46{,}344x + 1{,}378\,X_4 + 2{,}418\,X_6$$



By including seed lots 04 and 80 in the predictive equation, the equations corresponding to (3) and (6) become:-

$$y = 325.067 - 48.9391x \qquad (8)$$

and

$$z = -101.442 + 1.971\ W_4 + 2.319\ W_6 \qquad (9)$$

The analysis has emphasised the importance of exudation as a guide to the field emergence of seed lots of pea, and suggested that 4 in (10 cm) soil temperature and the number of hours below 50°F after planting were of importance in influencing the final percentage emergence. The final predictive emergence equation from the 1970 field data, obtained by combining equations (8) and (9) is therefore:-

$$y = 223.625 - 48.939x + 1.971\ W_4 + 2.319\ W_6 \quad (10)$$

It is this equation that will be tested using the 1971 field emergence data. It must be remembered that any figure obtained for y, must be transformed back to a percentage figure, and not used directly as an estimate of potential emergence.

The mean emergence results and weather variables obtained for the 1971 field plantings are shown in Table 23.

Three seed lots 59, 72 and 73, had high emergences at both planting dates, but seed lots 67, 03 and 11 showed increasing levels of emergence similar to those of an intermediate seed lot.

Unfortunately, the continuous soil temperature recorder was damaged at the start of the 1971 season, and the weather variable W_6, hours below 50°F after planting, could not be obtained. This necessitated modifying the predictive equation to allow for the omission of W_6.

Table 23. The exudation levels of the six seed lots used in the 1971 field experiment, with the mean % emergence values of the lots, and the weather variables recorded at the two planting dates.

Planting Date	Seed Lot						1 ft Soil Temp (^{O}F)	Air Temp ^{O}F	2 in Soil Temp (^{O}F)	4 in Soil Temp (^{O}F)	Rainfall (mm)
	67	03	59	11	72	73					
1	25	39	74	31	91	92	39.7	46	46	39	33.8
2	59	55	87	47	100	97	40.9	50	49	42	11.7
Exudation μmho cm^{-2}	192	212	117	207	137	122					

The modified equation corresponding to equation (9) was therefore:-

$$z = -62.074 + 1.307\ W_4 \quad P < 0.001 \qquad (11)$$

The final predictive equation to be used on the 1971 data was obtained by combining equations (8) and (11), giving

$$y = 262.993 - 48.9391x + 1.307\ W_4 \qquad (12)$$

Using this equation, and equation (8) which did not include any weather variables, the expected emergence values for the six seed lots at the two planting dates were calculated, and are presented, alongside the observed emergence data in Tables 24(a) and 24(b).

Reference to Tables 24(a) and (b) shows that neither of the two equations produced from the 1970 emergence data were very accurate in predicting the emergence, in 1971 of a new set of seed lots. However, it is apparent that the equation involving the weather variable W_4, predicts an emergence figure closer to the observed figure, than the equation relying upon exudation alone, for the three intermediate seed lots 67, 03 and 11. The inclusion of the weather variable has enabled a more realistic prediction of the emergence of intermediate seed lots, than exists at present, but it is still not accurate enough.

It is apparent in this case, therefore, that the predictive equation produced from the 1970 data did not give a very reliable indication of seed lots planted in 1971. However, Table 25 shows how a predictive equation could be used, not only to simply predict emergence of seed lots, but to

Table 24 The observed and expected % emergence values, calculated using the two predictive equations, for the six seed lots, at the two planting dates.

(a) Planting date 1.

Seed lot	Observed	Expected	
		Equation (8)	Equation (12)
59	74	100	97
72	92	100	97
73	91	99	92
67	25	85	70
11	31	81	64
03	39	79	62

(b) Planting date 2.

Seed lot	Observed	Expected	
		Equation (8)	Equation (12)
59	87	100	99
72	97	100	98
73	100	99	95
67	59	85	76
11	47	81	70
03	55	79	68

suggest what types of seed could be used in areas where the average soil temperatures in springtime are known.

Table 25. The predicted emergence of seed lots with different levels of exudation, planted in soils of different temperature, calculated from the final predictive equation evolved from the 1971 data.

Exudation (µmho cm^{-2})	32°F	34°F	36°F	38°F	40°F	42°F
150	59.7	62.4	65.2	67.6	70.2	72.8
175	51.9	54.7	57.5	59.9	62.5	65.1
200	45.6	48.3	51.2	53.5	56.1	58.7
225	39.8	42.5	45.3	47.7	49.3	52.1
250	34.7	37.4	40.2	42.4	45.2	47.8
275	30.0	32.7	35.5	37.9	42.5	43.1
300	25.8	28.5	31.3	33.7	36.3	38.9
325	21.0	24.6	27.3	29.7	32.3	34.9
350	18.2	20.9	23.7	26.1	28.7	31.3
375	14.9	17.6	20.4	22.8	25.4	27.9
400	10.9	14.4	17.2	19.6	22.2	24.8
425	8.8	11.4	14.2	16.6	19.2	21.8
450	5.9	8.7	11.5	13.9	16.5	19.1
475	3.4	5.9	8.8	11.2	13.8	16.4
500	0	3.5	6.3	8.7	11.3	13.9

Taking for example an emergence of less than 50% as a failure, a seed type with an exudation as low as 175 µmho cm^{-2} would have to be planted, in soils at 32°F, to obtain successful emergence. Similarly, any seed lot with an exudation of over 225 µmho would give an emergence failure, even if planted in soils where the 4 in (10 cm) soil temperature did not fall below 42°F for two weeks after planting.

DISCUSSION.

Previous investigations of a predictive nature in agronomy have concentrated on the influence of weather conditions, and certain plant characteristics, on the yield of crop plants. Black, (1970), found that the yield of wheat was closely associated with the number of adventitious roots per plant. Thompson (1969, 1970), investigating the role of weather in the production of soybean, regressed climate against yield, introducing a factor due to technology, and found that higher than average yields were associated with warmer than normal June temperatures, and above normal precipitation in July and August.

However, there have been few attempts to predict the emergence and establishment of crop plants, even though it would seem easier than predicting the yield of a crop after the complex interactions of a growing season. The predictive emergence equation, produced by Bradnock & Matthews (1970), did not take into account the influence of soil conditions at the time of planting, even though it had been demonstrated on several occasions that soil conditions were very influential. Nevertheless, their analysis demonstrated the importance of seed exudation, and indicated that seed weight did not play a major role in producing differences in field emergence between seed lots. Future investigations could therefore discount the variable, seed weight, without seriously detracting from the analysis.

The analysis reported here has emphasised the importance of exudation as a guide to field emergence, and suggested that the 4 in (10 cm) soil temperature, and the number of hours below 50°F after planting, were major factors influencing the final percentage emergence.

Although the statistical approach to studying the influence of weather on crops has been criticised (Milthorpe, 1965), Alcock, Lovett and Machin (1968), have suggested it is useful in indicating which weather variables are most important.

In this work the important weather variables influencing emergence have been detected using the statistical approach. Soil temperature was shown to be important and future work could therefore concentrate more on this measurement than on other weather variables. Alcock et al have commented, however, that soil temperature is a very complex factor to interpret since, particularly in the spring, it is correlated positively with radiation, grass minimum and air maximum temperatures, and negatively with wind speed, relative humidity and soil water content.

This simply emphasises, therefore, that the influence of soil conditions, and weather, on seed emergence is complex and that a statistical approach to the problem is probably the only one that can take into account a large number of the variables.

Hegarty (1971), investigating the relationship between field emergence and laboratory germination of carrots, went some way in overcoming the problem of the influence of soil conditions, by producing a predictive emergence equation based on the laboratory germination of the seed lots under stress conditions. In peas, Clark and Baldauf (1958) demonstrated that a 'cold test' can give a reliable indication of field emergence of some seed lots. By using the emergence of the carrot seed in a 10°C laboratory germination test, Hegarty acknowledged that a predictive equation should involve some factor relating to the stress conditions the seeds may encounter in the field. His final equation gave a reliable indication of the field emergence of carrot seeds in the soil conditions found in the East of Scotland during the early part of the growing season.

The final predictive equation computed in this work from the 1970 field emergence trials, did not give a very reliable indication of the field emergence of another set of seed lots of Dark Skinned Perfection, planted in 1971. This lack of predictive accuracy may be associated with experimental factors. Firstly, the dates of planting for 1971 were prior to those used for the 1970 model, and that model overestimated emergence in the early part of the season, even when it was checked on the two seed lots that were omitted from the original analysis. Secondly, the levels of exudation of the six seed lots used in 1971, were generally outside the range used in the 1970 model. Thirdly, it is unreasonable to assume that an accurate predictive equation can be produced when data from only one season, and one site, has been used.

The first reason, that of planting dates, could well be classed as an experimental error, but the second reason was unavoidable. The material was sent by a supplier, who supplied some of the 1970 seed, following a request for seed with differing levels of exudation, and it may well be an indication that the overall level of seed quality is improving.

Even though the final predictive equation proved unreliable, there were indications during the course of the work, that an accurate predictive equation could be developed. The equation produced by using six of the eight 1970 seed lots, proved extremely useful in predicting the emergence of the remaining two lots. This presumably succeeded because the levels of exudation, and weather conditions were inside the bounds of those that were used to form the predictive equation. It is possible, therefore, that if a wide enough range of levels of exudation and weather conditions can be included, a predictive equation could be formed that would prove to be a reliable indication of the field emergence of seed lots.

The concept of a predictive equation for seed emergence, in which weather variables are included might well be useful, and may eventually be of practicable value. Areas of the country in which it is known that soil conditions, in this instance 4 in (10 cm) soil temperature, will predispose many seed lots to pathogenic attack, could well be designated as areas where only top quality seed should be sown. These seed lots, possessing low levels of exudation, could well be used therefore in areas, and at planting times, when they were particularly needed, and not used in areas where inferior seed lots would suffice.

The analysis of the data from the emergence trials, although indicating the weather variables, and seed condition factors, most associated with emergence failures, was unable to produce a predictive emergence equation that was reliable in the subsequent season. Nevertheless, the statistical approach has been shown to be encouraging, and it may be that a more comprehensive coverage of seed conditions, and weather conditions, will prove more rewarding.

GENERAL DISCUSSION

GENERAL DISCUSSION

Disease forecasting has nearly always been based on the influence of the environment on the pathogen and its epidemiology. In the disease situation studied in this work it appears that the major effect of the environment is not on the pathogen, but on the host. Nevertheless, the empirical objective, the forecasting of the likelihood of high levels of disease, in this case emergence failure, is the same as in all disease forecasting.

Miller (1959) described disease forecasting as a study in applied epidemiology, but in this work, it seems unlikely that a change in the pathogenicity or incidence of the fungus, Pythium ultimum, is responsible for the differences in emergence that were observed in different conditions. It is the physiological changes that occur within the seeds, as a result of the environmental conditions, that are the probable cause of the differences in emergence.

The differences in emergence between the seed lots were most apparent during the early sowing dates of the field experiments. The results of these experiments agreed with the findings of Harper, Landragin and Ludwig (1955a) and Wellington (1962) in that the largest discrepancies between laboratory germination and field emergence occurred when the period of establishment was prolonged by low temperatures. However, certain of the seed lots used here, notably those classed as good, managed to achieve high levels of emergence, even after the delay in emergence caused by low temperatures. The most interesting responses to temperature were exhibited by the intermediate seed lots, whose levels of emergence increased as temperatures rose during the season.

Unfortunately, field conditions did not allow the influence of soil moisture on emergence, to be examined in the field, but in laboratory soil emergence tests, soil moisture was shown to have a pronounced effect on seed emergence. Once again, however, some seed lots responded more than others, in so far as they showed marked reductions in emergence, under wet and cold conditions.

The varying emergence responses of the different seed lots to the different temperature and moisture conditions, indicated that the conditions did not simply influence the fungus which causes pre-emergence seed mortality. The differences in emergence appeared to result from the complex interaction of the host, the pathogen and the environment.

The profound changes in the physiology of the seeds after low temperature and high substrate moisture treatments, indicated that the interaction between the host and the environment was very important. Furthermore, the extent to which the physiology of the seed lots was affected, was related to the ability of the seed to perform in the field under adverse conditions. The most important changes that were found as a result of environmental conditions occurred in the respiration of the seeds.

Sherwin and Simon (1969) demonstrated that under wet conditions <u>Phaseolus</u> spp seeds accumulated, temporarily, high levels of ethanol, and on removal from the wet conditions they were found to initially evolve high levels of carbon dioxide. In general, similar responses in peas have been noted in this work but seed lots were found to respond in different ways to conditions of high substrate moisture and low temperature. Seed lots showing high soil emergence in all conditions were capable of maintaining relatively high levels of oxygen uptake until extremely moist conditions were encountered, when they adopted vigourous levels of anaerobic respiration. The important feature of these high moisture conditions was most probably the low oxygen content of the substrate.

Spragg and Yemm (1959) and Kolloffel (1967) found that the presence of an intact seed coat in peas limited gaseous diffusion and Sherwin and Simon (1969) produced evidence which suggested that the presence of the testa on bean seeds, resulted in lower levels of oxygen uptake during wet conditions; but the differences in the ability of seed lots to maintain oxygen uptake levels in conditions of high substrate moisture may not be solely related to differences in seed coats.

The influence of substrate conditions on the seeds was not limited to their levels of respiration and ethanol accumulation. The conditions, particularly low temperature, markedly influenced the exudation levels of the seeds. Conditions of high substrate moisture also led to high levels of exudation most probably through providing anaerobic conditions. A similar observation was made by Brown and Kennedy (1966), in soy bean seeds, who showed that low oxygen concentrations increased the levels of exudation. However, the influence of conditions on exudation was the same for all seed lots suggesting that changes in exudation did not completely explain the differences in the extent to which emergence of seed lots was affected by adverse conditions.

Although the conditions of the sowing medium were found to influence the physiological state of the seeds, the conditions were never so severe as to reduce the viability.

However, the pretreatments to which the seeds were subjected had a significant influence on their abilities to emerge in unsterilized soil. Wet and cold pre-treatments reduced emergence of some seed lots and *Pythium ultimum* was readily isolated from the seeds that failed to emerge. This suggested, therefore, that the changes in the physiological state of pea seeds, as a result of conditions of high substrate moisture and low temperature, predisposed some seed lots to pathogenic attack.

The pathogenic component in the host/pathogen/environment interaction is therefore all important since without this factor, the majority of seeds in these experiments would have been capable of withstanding adverse conditions, even though they had suffered short-term alterations to their physiological state.

The respiratory response of seed lots to sowing conditions is probably the most fundamental observation made, in the work reported here. Maintaining an adequate supply of energy may well be the means by which seeds can resist infection and retain cell contents. Any environmental condition that interferes with the availability of energy in seeds could result in the breakdown of the normal mechanisms of resistance and thus lead to infection and subsequent mortality.

Apparently, the seed lots that are particularly vulnerable to adverse environmental conditions are unable to maintain a supply of energy at low temperatures and in the relatively anaerobic conditions associated with high moisture.

All the seed lots investigated in this work were of the same cultivar. Therefore, it would appear that the differences observed between the seed lots were associated with some feature of their production, which in some way affects their metabolic capabilities.

Changes in predisposition to pathogenic attack would seem to be the response of seeds to environmental conditions which ultimately results in emergence failures. The relationship between soil conditions and seed quality has been clarified and an explanation linking soil conditions and changes in predisposition based on physiological responses has been forwarded. This improved understanding may assist attempts to make accurate predictions of the field emergence of pea seeds.

An attempt was made to predict the field emergence of pea seeds using multiple regression techniques. Despite the failure of the predictive equation produced to give accurate predictions of emergence, the way in which such an equation could be used in agricultural management was illustrated.

This approach to agricultural management would be greatly enhanced by improved meteorological forecasting. The role of meteorological forecasting in agricultural management has been discussed by Duckham, who was quoted by Gloyne (1972) as follows: "the function of forecasting is to enable the decision-maker to take better informed positive action, evasive action or inaction."

An objective of the work of this thesis has been to gain a clearer understanding of the influence of the environment on pea seed emergence, in order to provide a firmer foundation on which a decision-maker, the grower, can base his actions.

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APPENDICES

Appendix I

Correction Factors for use with the Gilson Differential Respirometer

Oxygen Uptake.

The multiplication factor, necessary to convert the relative volumes of gas measured on the instrument to standard conditions, microlitres of dry gas at 760 mm Hg, and 273° Absolute was calculated as follows:-

$$\frac{(273)\ (Pb)}{t+273)\ (760)}$$

where, t = manometer temperature and Pb = operating barometric pressure.

The value for t is very rarely ambient temperature, and was taken as the temperature across the manometers during the period of the experiment.

This formula does not include a correction for water vapour. However, if a measured amount of water is added to the reference flask, this not only ensures that the gas volumes on the active and reference sides of the system are balanced, but also that they are both saturated with water vapour during the experiment.

Carbon Dioxide Evolution.

Gregory and Winter (1965) pointed out that "in constant-pressure respirometers, in which the cylinder holding the variable gas volume is not immersed in the water bath, the entire gas volume increase (or decrease) will be at a different temperature from the water bath. In this case accurate data reduction requires a knowledge of the temperature of both the reaction fluid (water bath temperature) and the altered gas space (similar to air temperature surrounding the cylinder)."

They suggested a complete conversion equation, and three simplified versions. In the particular case of a respirometer with the variable gas volume and the fluid at different temperatures, as is the case for the Gilson Differential Respirometer, only the complete equation was accurate.

The multiplication factor (x) was therefore derived as follows:-

$$x = \Delta Vg \; \frac{P - Pwt}{P't} \left[\frac{T_1 + TV_f(\alpha x - \Sigma^n i{=}1 \; a_i \; \alpha_i)}{Vgm} \right]$$

Where x = total amount of gas being measured, expressed as μP at standard condition.

ΔVg = observed change in volume of respirometer,

P = total gas pressure within the respirometer in mm Hg

Pwt = vapour pressure of water at temperature t in mm Hg

p^1 = standard pressure, 760 mm Hg,

t = temperature of variable gas volume when different from T, $^{\circ}$K,

T_1 = standard temperature, 273°K,

T = temperature of water bath, $^{\circ}$K,

V_f = volume of fluid in respirometer, in μl.

αx = Bunsen coefficient of assayed gas x, at temperature T, ml gas dissolved/ml water at 760 mm Hg pressure of gas X.

ai = fraction of each dry gas i initially present in respirometer.

αi = Bunsen coefficient of each gas i in respirometer, at temperature T, ml gas dissolved/ml water at 760

αi = Bunsen coefficient of each gas i in respirometer, at temperature T, ml gas dissolved/ml water at 760 mm Hg pressure of gas i

Vgm = mean gas volume of respirometer (piston one-half compressed), µl.

Carver and Gloyne (1971) discuss in more detail precautions necessary when using the Gilson differential respirometer.

REFERENCE

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Appendix II

Gas-Liquid Chromatography Technique

A single column flame gas chromatograph, coupled with an integrated recorder was employed, wherein peak height and peak area were obtainable as detector response. A stainless steel column ($\frac{1}{8}$" o.d. x 2 m) containing Carbowax 100% on AW-DMCS Chromosorb W (100-120 mesh) was used throughout the experiments.

The pressures of the hydrogen and air were 15 and 25 lbs/sq in respectively, and the carrier gas, nitrogen, was maintained at a pressure of 11 lbs/sq. in. The detector temperature was 120°C.

When 2 μl samples of the homogenized seeds were injected, satisfactory peak heights were obtained when the sensitivity of the chromatograph was set at 20 x 1.

The amounts of ethanol present in the samples were calculated by comparing their peak heights to those obtained from 2 μl injections of 0.5% M. ethanol.

Appendix III

Gaseous Exchange in a Nitrogen Atmosphere

The carbon dioxide output, in µl/h/gm d.w. in air was compared to the output when the seeds were subjected to anaerobic conditions in 100% nitrogen gas.

The response of the replicates is shown in Table (i).

Table (i) The CO_2 output of the seed lots under nitrogen, expressed as the percentage increase over their output in air.

Replicate	Seed lot				
	39	04	03	54	20
I	57	31	65	149	14
II	86	10	85	36	22
III	73	31	60	59	-16
IV	39	17	10	85	-45
V	40	41	7	20	-5
VI	67	34	12	62	19
VII	94	15	44	27	-20
VIII	37	21	37	21	-8
IX	58	52	56	19	-10
X	85	37	12	40	15
XI	94	43	24	20	10
XII	86	25	12	54	5
XIII	95		20		7
XIV	72				
XV	67				
MEAN	+70	+29	+34	+49	-2

The blank spaces indicate missing replicates.

Appendix IV

Emergence data from the 1970 field experiment

Table (ii) Emergence for all four replicates of nine planting dates in the Nursery site

Seed Lot	Planting date								
	1	2	3	4	5	6	7	8	9
39	60.67	43.85	81.87	81.87	68.03	71.57	66.42	78.46	60.67
	59.34	60.67	78.46	81.87	64.90	78.46	68.03	69.73	68.03
	69.73	39.23	75.82	71.57	73.57	78.46	64.90	68.03	71.57
	73.57	53.13	90.00	69.73	75.82	81.87	81.87	75.82	71.57
03	50.77	30.00	58.05	75.82	75.82	75.82	56.79	78.46	71.57
	38.06	47.29	68.03	69.73	71.55	73.57	69.73	73.57	71.57
	48.45	31.95	68.03	71.57	81.87	81.87	78.46	71.57	73.57
	46.15	42.71	62.03	62.03	81.87	69.73	62.03	78.46	68.03
47	56.79	43.85	62.03	81.87	71.57	69.73	59.34	63.43	66.42
	41.55	56.79	69.73	73.57	81.87	68.03	53.13	71.57	68.03
	50.77	45.00	64.90	68.03	75.82	78.46	59.34	69.73	75.82
	50.77	46.15	63.43	64.90	75.82	71.57	51.94	78.46	56.79
04	27.97	29.33	45.57	58.05	66.42	62.03	68.03	54.33	53.13
	20.57	39.82	54.33	62.03	54.33	58.05	53.13	60.67	58.05
	29.33	16.43	59.34	43.85	59.34	59.34	51.94	60.67	48.45
	34.45	29.33	53.13	53.14	63.43	81.87	56.79	56.79	42.71
54	33.21	34.45	58.05	69.73	62.03	60.67	69.73	64.90	71.57
	34.45	38.06	59.34	78.46	60.67	60.67	56.79	69.73	58.05
	38.06	23.58	59.34	66.42	73.57	75.82	58.05	59.34	62.03
	1.00	29.33	64.90	64.90	69.73	64.90	59.34	68.03	63.43
80	1.00	1.00	11.54	25.10	14.18	23.58	29.33	29.33	25.10
	5.74	1.00	16.43	27.97	21.97	29.33	34.45	31.95	21.95
	1.00	1.00	14.18	20.27	26.57	23.58	23.58	25.10	23.58
	1.00	1.00	16.43	16.43	20.27	31.95	18.43	20.27	16.43
21	1.00	1.00	16.43	14.18	14.18	23.58	23.58	20.27	30.66
	1.00	1.00	16.43	21.97	20.27	21.97	23.58	21.97	23.58
	1.00	8.13	16.43	14.18	25.10	21.97	23.58	23.58	20.27
	1.00	1.00	1.00	25.10	18.43	16.43	21.97	25.10	14.18
20	11.54	8.13	27.97	35.67	27.97	29.33	46.15	29.33	39.23
	1.00	11.54	25.10	34.45	32.45	25.10	35.67	36.87	38.06
	8.13	8.13	21.97	34.45	31.95	46.15	31.95	36.06	27.97
	11.54	14.18	34.45	29.33	38.06	31.95	38.06	29.33	27.97

In Tables (ii), (iii) and (iv) the field emergence of the seed lots are presented after correction for their differing viabilities and after arc sin transformation.

Table (iii) Emergence for all four replicates of nine planting dates in the Field site.

Seed lot	Planting date								
	1	2	3	4	5	6	7	8	9
39	73.57 69.73 64.90 68.03	78.46 78.46 69.73 71.57	73.57 78.46 73.57 73.57	78.46 78.46 68.03 81.87	78.46 81.87 81.87 78.46	64.90 68.03 63.43 90.00	75.82 78.46 90.00 80.03	78.46 75.82 90.00 78.46	71.57 75.82 81.87 68.03
03	53.13 54.33 42.71 53.13	59.34 68.03 53.13 54.33	66.42 66.42 62.03 66.42	78.46 66.42 69.72 69.72	90.00 69.73 75.83 73.57	71.57 73.57 75.82 78.46	64.90 64.90 73.57 73.57	78.46 75.82 78.46 73.57	66.42 75.82 71.57 63.43
47	62.03 66.42 50.70 53.13	64.90 75.82 63.43 64.90	75.82 69.73 62.03 71.57	73.57 81.87 75.82 69.73	73.57 81.87 73.57 81.87	66.42 78.46 64.90 71.57	62.03 63.43 64.90 62.73	71.57 75.82 71.57 75.82	71.57 75.82 66.42 90.00
04	29.33 29.33 27.97 22.79	40.40 38.06 36.87 38.06	46.15 56.79 42.71 40.40	62.03 71.57 62.03 73.57	57.33 56.79 58.05 48.45	56.79 59.34 58.05 51.94	46.15 59.34 60.67 56.17	66.42 64.90 62.03 71.57	54.33 54.33 62.03 60.67
54	40.40 26.57 39.23 33.21	58.05 53.13 43.85 55.55	60.67 62.03 53.13 54.33	68.03 71.57 68.03 73.57	69.73 75.82 69.73 74.66	68.03 69.73 71.57 69.73	62.03 63.43 59.34 60.00	71.57 68.03 69.73 78.46	64.90 66.42 60.67 68.03
80	01.00 01.00 01.00 08.13	01.00 01.00 01.00 01.00	20.27 16.43 11.54 01.00	23.58 31.95 29.33 20.27	21.97 23.58 31.95 25.10	34.45 26.57 29.33 25.10	30.66 34.45 31.95 30.00	35.67 38.06 42.71 42.71	18.43 14.18 27.97 20.27
21	11.53 01.00 01.00 01.00	01.00 01.00 01.00 08.13	11.54 05.74 01.00 01.00	18.43 26.57 23.58 16.43	20.27 21.97 31.95 25.10	23.58 31.95 29.33 25.10	29.33 20.27 33.21 25.10	33.21 40.40 34.45 38.06	14.18 26.57 18.43 16.43
20	16.43 08.13 08.13 14.18	18.43 14.18 18.43 11.54	26.57 29.33 18.43 18.43	39.23 29.33 35.67 30.66	34.45 35.67 39.23 38.06	41.55 36.87 31.95 31.95	40.40 21.97 49.60 35.67	40.40 47.29 53.13 43.85	33.21 31.95 25.10 26.57

Table(iv) Emergence for all four replicates of nine planting dates in the Sword site

Seed lot	Planting date							
	1	2	3	4	5	6	7	8
39	71.57	73.57	73.57	73.57	73.57	60.67	54.33	71.57
	59.34	71.57	71.57	71.57	58.05	59.34	66.42	75.82
	68.03	63.43	71.57	75.82	66.42	39.23	59.34	90.00
	81.87	68.03	78.46	69.73	69.73	54.33	58.05	71.57
03	50.77	50.77	63.43	64.90	63.43	63.43	45.00	62.03
	42.71	53.13	64.90	71.57	56.79	55.55	50.77	58.05
	43.85	56.79	59.34	69.73	71.57	50.77	56.79	62.03
	58.05	49.60	71.57	71.57	73.57	55.55	50.77	68.03
47	63.43	63.43	66.42	81.87	62.03	56.79	53.13	63.43
	50.77	59.34	66.42	66.42	53.13	60.67	48.45	71.57
	59.34	64.90	64.90	75.82	64.90	47.29	26.57	63.43
	59.34	66.42	68.03	78.46	64.90	59.34	49.60	64.90
04	33.21	40.40	50.77	55.55	51.94	55.55	55.55	51.94
	36.87	42.71	45.00	43.85	46.15	42.71	55.55	56.79
	42.71	40.40	45.00	55.55	63.43	45.00	54.33	51.94
	33.21	48.45	56.79	62.03	64.90	50.77	53.13	60.67
54	45.00	39.23	54.33	73.57	62.03	54.33	54.33	56.79
	47.29	50.77	63.43	56.79	58.05	51.94	35.67	66.42
	49.60	58.05	62.03	71.57	68.03	51.94	55.55	64.90
	48.45	58.05	63.03	60.67	69.73	43.85	55.55	60.67
80	1.00	8.13	16.43	21.97	21.97	20.27	18.43	29.33
	8.13	11.54	14.18	14.18	16.43	21.97	25.10	35.67
	1.00	8.13	20.27	20.27	25.10	14.18	21.97	30.66
	11.54	8.13	20.27	23.58	18.43	18.43	16.43	27.97
21	8.13	8.13	20.27	14.18	18.43	18.43	27.97	26.57
	1.00	20.27	11.54	18.43	16.43	18.43	21.97	23.58
	1.00	8.13	11.54	18.43	20.27	16.43	16.43	27.97
	8.13	11.54	18.43	29.33	23.58	14.18	20.27	36.87
20	8.13	20.27	35.67	30.66	32.45	38.06	21.97	35.67
	8.13	1.00	25.58	34.45	23.58	31.95	41.55	43.85
	11.54	20.27	36.87	30.66	26.57	23.58	35.67	18.43
	21.97	27.97	36.87	36.87	38.06	21.97	42.71	35.67

Appendix V

Weather variables selected for regression analysis in Section IV

Table (v) Weather variables for each planting date in 1970.

Planting Date	1 ft temp °F	Air temp °F	2 in temp °F	4 in temp °F	Rainfall (mm)	Hours below 50°F
1	38.3	46	44	36	13.1	335
2	39.1	45	47	39	5.1	265
3	44.9	50	55	44	0.2	174
4	49.0	59	63	49	27.2	59
5	51.6	57	66	50	3.3	32
6	52.9	60	67	52	4.1	16
7	61.5	68	82	57	18.9	0
8	59.8	61	66	53	15.6	0

FURTHER STUDIES ON RAPID SEED EXUDATE TESTS INDICATIVE OF POTENTIAL FIELD EMERGENCE

by

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INTRODUCTION

TAKAYANAGI and MURAKAMI (1968) have reported that seeds of rape, *Brassica napus* L., with no germinability exuded considerably more sugars when soaked in water than did seeds of good germinability, and a rapid exudation test based on this phenomenon was suggested. The relationship between laboratory germination and seed exudation has been recognised for many years (HOTTES and HUELSON, 1927; PRESLEY, 1958). Moreover, the use of seed exudates in seed testing has been extended to provide a guide to emergence in the field. Some seed stocks of certain crops contain large numbers of seeds that are viable in laboratory tests but which fail to emerge in the field. The pre-emergence failure has been attributed to soil borne fungi (TATUM, 1954; THOMAS, 1960) and in at least two crops, maize (TATUM, 1954) and castor beans THOMAS, 1960) the emergence of seed stocks in soil has been found to be negatively correlated with the readiness with which seeds from the stocks exuded materials into soak water.

More recently a similar relationship between soil emergence and seed exudation was found for seed stocks of wrinkled-seeded peas *Pisum sativum* L. (MATTHEWS and WHITEBREAD, 1968) and for stocks of French beans, *Phaseolus vulgaris* L. (MATTHEWS and BRADNOCK, 1968). The exudation of seeds into soak water was determined by measuring the electrical conductivity of the soak water after 24 hours, a method that was previously used by THOMAS (1960) for castor beans. In peas (MATTHEWS and WHITBREAD, 1968) the electrical conductivity of soak water was shown to be negatively correlated with laboratory germination and positively correlated with the predisposition of the viable seeds to pre-emergence mortality.

The work on peas has been developed further. Firstly the value of electrical conductivity as an indicator of the amino acid and sugar content of soak water was examined and secondly a modification of the Japanese rapid seed test was tried for peas.

METHODS AND MATERIALS

Exudate analyses

Four replicates of twenty seeds of each of ten stocks of peas were steeped for 24 hours at 15°C in 200 ml of de-ionized water after which time the total electrical conductivity of the soak water was measured with a Mullard conductivity bridge (MATTHEWS and WHITBREAD, 1968). The soluble carbohydrate content, both reducing and nonreducing sugars, of the soak water was determined by a method (DUBOIS et al, 1951) involving phenol and concentrated sulphuric acid and the amino acid content by a ninhydrin method (YEMM and COCKING, 1955). The seed stocks used were all commercially acceptable having a high laboratory germination but some were known to give lower field emergence counts than others (MATTHEWS and WHITBREAD, 1968).

Germination and Emergence tests

Six stocks of peas were examined, three of the cultivar Witham Wonder and three of Kelvedon Wonder. All the stocks had a laboratory germination greater than the declarable minimum of 80 % (Table 1) as determined at licensed seed testing station in the U.K. Field emergence percentages were obtained from four replicates of 50 seeds of each stock in a field sowing at Stirling, Scotland in March 1969.

Table 1. Comparison for 6 stocks of peas of laboratory germination, field emergence and Clinitest *reaction.*

Cultivar	Stock	% Lab Germination	% Field Emergence	Colour after *Clinitest*	Concentration of glucose in mg/ml
Witham Wonder	1	95	61	blue	0
	2	94	43	green	0.5 mg/ml
	3	90	13	yellow	1 mg/ml
Kelvedon Wonder	1	98	74	blue	0
	2	93	40	green	0.5 mg/ml
	3	93	33	green	0.5 mg/ml

Rapid exudation test

Exudates were produced from each of the 6 stocks that had been sown in the field by soaking 20 seeds in 100 ml of distilled water at 15° C. After 24 hours 1 ml of soak water was mixed with 0.1 ml invertase concentrate to convert the sucrose into glucose and fructose. A *Clinitest* reagent tablet was added to each tube and the mixture was shaken 15 seconds after effervescence had stopped. The colours of the final solutions were compared with standard colours and an assessment of the concentration of glucose present in the solutions was made. These reagent tablets are used in clinical tests on human urine.

RESULTS

Fig. 1 shows the highly significant regression between total electrical conductivity and soluble carbohydrate content, and between total electrical conductivity and amino acid content for the pea seed soak water. The concentrations of soluble carbohydrates present were about twenty times greater than the amino acid concentrations and showed less variations about the regression line, than did amino acids, particularly at the higher concentrations.

The concentration of glucose in soak water as determined by the rapid test was compared with the mean field emergence percentage for each of the 6 stocks (Table 1). Table 1 shows that within both cultivars the concentration of glucose detected in the modified seed soak water was an indication of their field emergence.

DISCUSSION

The total electrical conductivity of seed soak water which is readily determined can be used as an indication of the soluble carbohydrate and amino acid content of the water. Analyses of both sugars and amino acids are time consuming and as has been previously suggested (MATTHEWS and BRADNOCK, 1968) routine exudation tests using electrical conductivity would be more convenient in most seed testing laboratories. Furthermore, as the results of this work show the total electrical conductivity of seed steep water can be used as an indication of the soluble carbohydrate and amino acid content of the water, confirming the suggestion (MATTHEWS and BRADNOCK, 1967) that ready exudation when present in seeds is found for several soluble seed constituents and is a measure of a common attribute of seeds.

In the discussion of the papers at the Fifteenth International Seed Testing Congress, MURAKAMI (1969) said that success with urine sugar analysis was

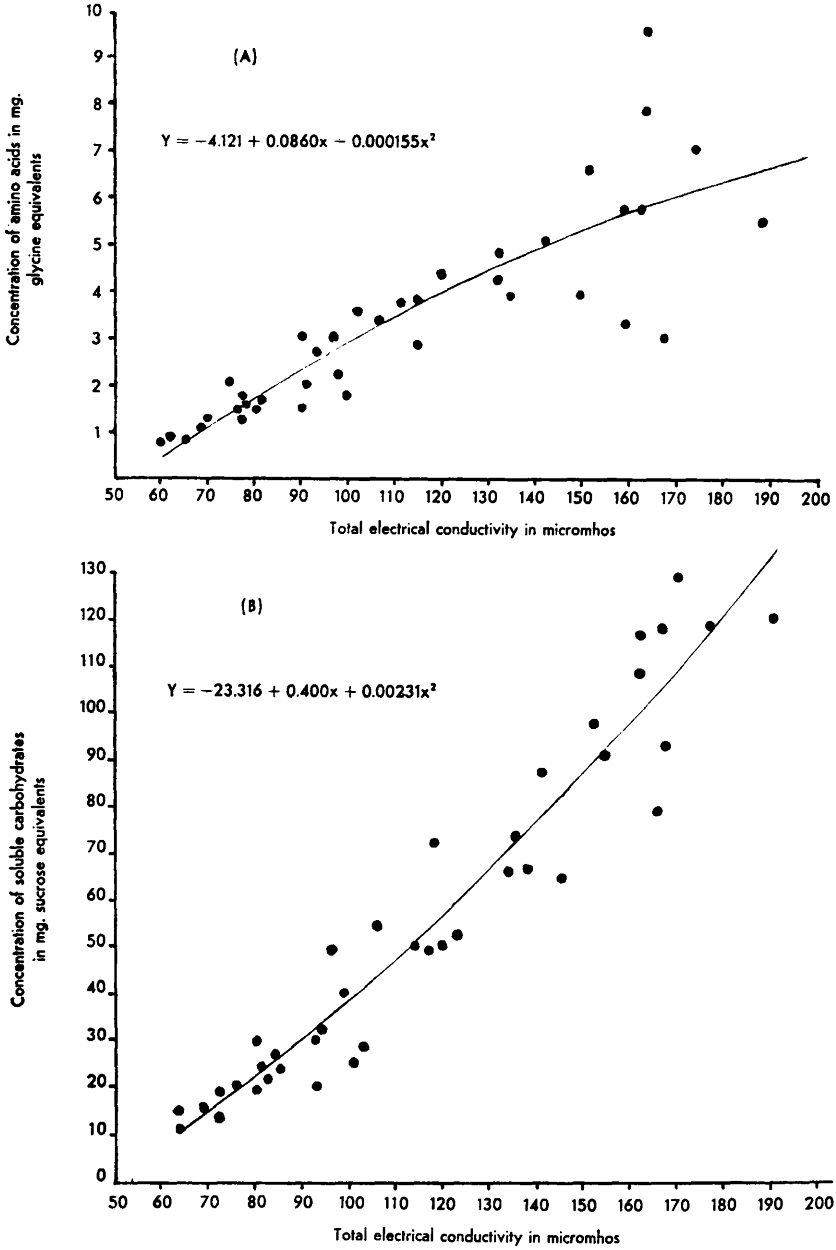

Fig. 1. Relationship between the total electrical conductivity of pea seed soak water and (A) its amino acid content and (B) its soluble carbohydrate content.

limited to testing for seed viability in species like rape, cabbage, and radish seeds whose seed exudates contain predominantly glucose. The simple modification of this test by the addition of invertase to exudate now enables the use of urine sugar tests on species whose seed exudate is predominantly sucrose and thus the potential usefulness of this type of test is considerably increased.

SUMMARY

The electrical conductivity of pea seed steep water is shown to be highly indicative of both amino acid and soluble carbohydrate content of the water.

A rapid sugar analysis for seed exudates is described for peas and is shown to be indicative of the potential field emergence of seed stocks. The enzyme invertase is used to convert the exuded sugar sucrose into glucose and fructose which can then be tested conveniently with urine sugar analysis tablets.

RÉSUMÉ

Étude complémentaire d'essais rapides indicatifs du pouvoir de levée en pleine terre sur exsudats de semences

Il est montré que la conductivité électrique de l'eau de trempage des pois est hautement significative et pour la teneur en acides aminés et pour la teneur en hydrates de carbone solubles.

On décrit une méthode rapide d'analyse du sucre dans des exsudats des semences des pois. Il est démontré qu'elle constitue un indice du pouvoir de levée des stocks de semences. On utilise l'enzyme invertase pour transformer le sucrose exsudé, en glucose et fructose, lesquels peuvent alors être analysés facilement à l'aide de tablettes pour l'analyse du sucre d'urine.

ZUSAMMENFASSUNG

Weitere Studien über Schnellprüfungen von Saatgutexudaten zur Anzeige des Feldaufganges

Die elektrische Leitfähigkeit des Einweichwassers von Erbsen zeigt nachweislich sehr gut den Gehalt des Wassers an Aminosäure und von löslichen Kohlenhydraten an.

Es wird eine Schnellanalyse der Saatgutexudate von Erbsen beschrieben und dargelegt, wie dadurch der Feldaufgang von Saatgut angezeigt werden kann. Das Enzym Invertase wird benutzt, um den ausgetretenen Rohrzucker in Glukose und Fruktose zu verwandeln, welche dann in der üblichen Weise mit Urinzuckerprüfungstabletten bestimmt werden können.

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The effects of variations in ambient temperature on the Gilson differential respirometer

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The effects of variations in ambient temperature on the Gilson differential respirometer

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Anomalous results were obtained during extensive work on pea seed respiration, using a Gilson differential respirometer (Gilson, 1963). Readings inconsistent with the general respiration pattern appeared to be associated with fluctuations in ambient temperature.

A close examination of the design of the Gilson respirometer indicated that fluctuations in ambient temperature might well produce inaccurate readings. Experiments were conducted to verify this possibility. In view of the large number of Gilson respirometers now in operation, we feel it important to report our findings.

Methods

A fourteen-station Gilson respirometer and two eight-station Warburg respirometers were set up using clean empty flasks and normal operating procedures (Figure 1).

The flasks were washed in sulphuric acid rinsed in distilled water, vacuum dried, and 3 ml of sterile water was added to each flask. During the first hour all three water baths were maintained at 20°C, with an ambient temperature outside the baths of 20°C. After one hour the Gilson Respirometer and one Warburg Respirometer were subjected to a drop in ambient temperature outside the water baths of 5°C (20 → 15°C). The second Warburg respirometer remained at an ambient temperature of 20°C. Any fluctuations in volume of gas in the respirometers were noted.

A second experiment was conducted on the Gilson Respirometer under different conditions on the recommendation of the Gilson Co. Three ml of sterile water were added to each sterile flask, and the reference flask contained a measured amount of water so that the gas volumes on the active and the reference sides of the respirometer were equal. Again any fluctuations caused by a drop in ambient temperature of 5°C were noted.

Results

During the first hour no changes in volume of gas were detected in any of the three respirometers. However when ambient temperature fell 5°C the Gilson Respirometer recorded an apparent uptake of 25 μl of gas. This apparent uptake did not occur in the Warburg subjected to the 5°C change or in the Warburg that remained in the constant ambient temperature.

When the volumes of gas were balanced the apparent uptake was reduced to 12 μl, when ambient temperature fell 5°C.

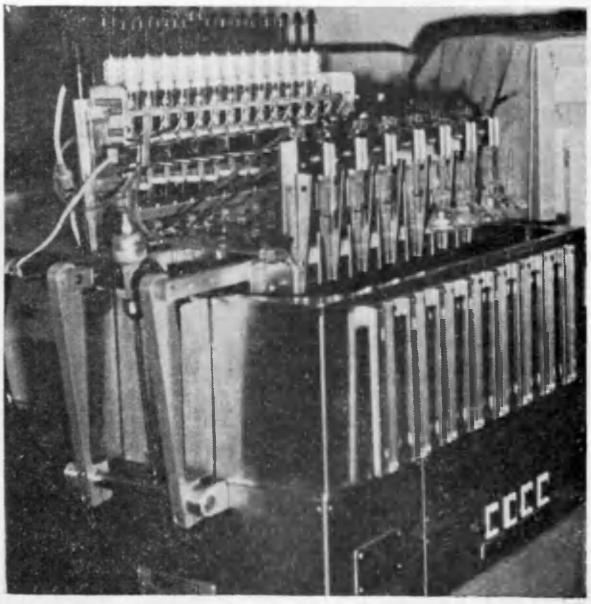

Figure 1.

Conclusions

The design of the respirometer in which different parts of the enclosed gas volumes are at different temperatures, some being in air and some being in the water bath, makes it difficult to decide which temperature to use for conversion of readings to S.T.P. Gregory and Winter (1965) suggested that the temperature of the variable gas volume should be used. This then allowed for accurate correction of data only if the variable gas volume did not fluctuate in temperature. It has been demonstrated here that, even when the system is balanced, an apparent uptake of 12 μl can occur in one hour if the ambient temperature falls 5°C. Such a fall in temperature can quite commonly occur in laboratories unless the experimenter is aware of its effects and can take precautions to minimize temperature fluctuations.

It is apparent that great care must be taken when conducting experiments using a Gilson Respirometer, especially if low levels of respiration are expected. If the following precautions are taken when using a Gilson Respirometer greater accuracy will be achieved. Firstly, correction using a thermoblank, as the thermobarometer is used in the Warburg system, will allow for fluctuations in ambient temperature. Secondly, care should be taken to minimize temperature fluctuation by avoiding direct sunlight, heaters or air draughts. Ideally the instrument should be used in a constant temperature room.

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The help of Dr S. Matthews of the Department of Biology is greatly appreciated.

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