AFRC INSTITUTE OF HORTICULTURAL RESEARCH, EAST MALLING

1988 PLACEMENT REPORT

STRAWBERRY BREEDING

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INTRODUCTION TO PLACEMENT 1988

East Malling Research Station is situated in the South-East of Great Britain, where much of the country's fruit is grown. It concentrates it's efforts on improving fruit varieties, fruit storage and propagation of fruit trees and woody ornamentals. The station was founded in 1913 as the Fruit Experimental Station of Wye College, Ashford, Kent. It became independent in 1920 under the official designation "The Kent Incorporated Society For Promoting Experiments In Horticulture". On the 1st of July 1987 the station was renamed the "Institute of Horticultural Research" funded by the Ministry of Agriculture Fisheries and Food, the Department of Education and Science, and external sources.

I worked in the Breeding and Genetics Department on strawberries. The aim of this department is to introduce improved varieties of apples, pears, cherries, plums, strawberries, raspberries and new rootstocks for top fruit.

STRAWBERRY BREEDING

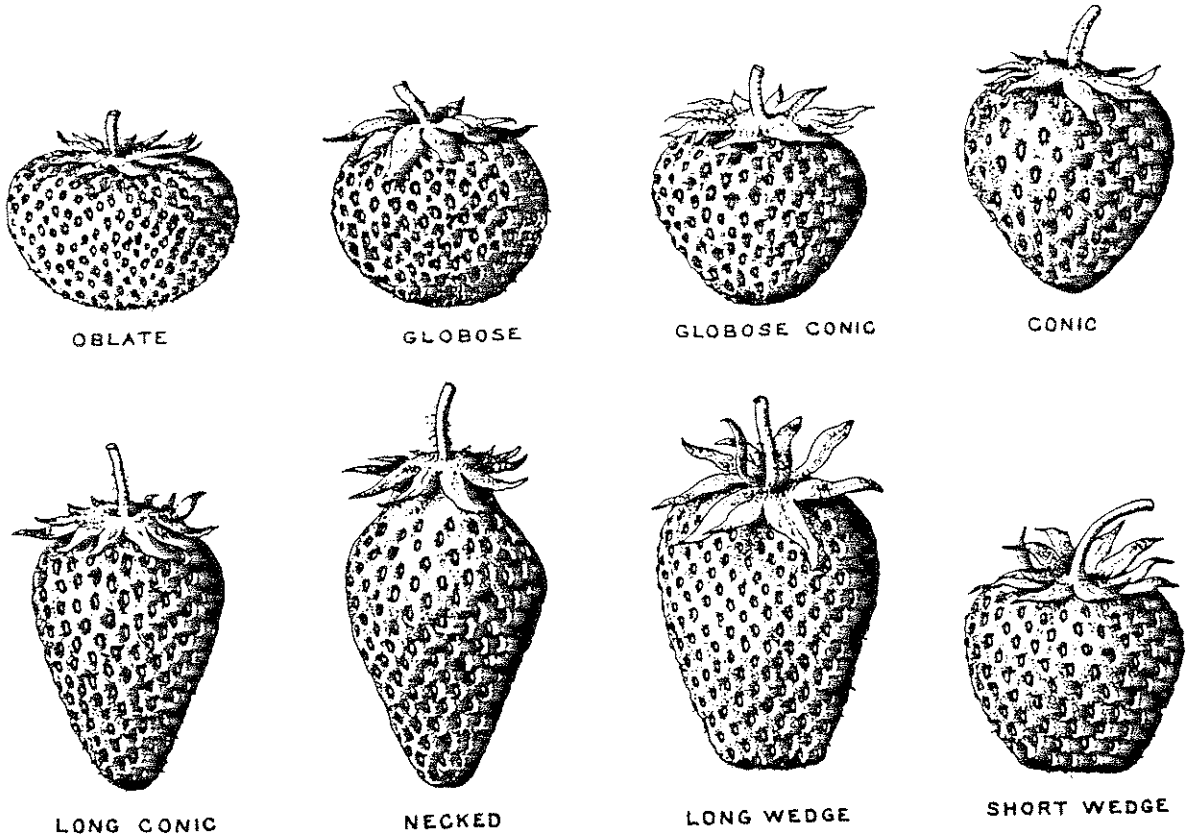
The breeder aims to improve a wide range of characteristics and qualities in strawberries. The strawberries must travel well so as to arrive in a satisfactory and marketable state at the retailers. For this they need to have a strong skin and firm flesh. The consumers and retailers also require the strawberries to be a suitable size and shape ie. 25mm - 45 mm in diameter and preferably conic or wedge shape (figure 1). Other characteristics that the strawberries need include an attractive appearance; they must be of good colour, preferably shiny and not blotchy, the

texture should be firm; moist but not slimy. Flavour is a very important feature in selecting strawberries and is assessed by tasting panels and the breeders themselves.

The principle aim of the strawberry breeding programme at IHR, East Malling is to produce four or five high quality varieties which will crop consecutively over a five month season. This period runs from May to October, with the earliest fruit produced under protection in the form of polythene tunnels.

A ten to twelve year breeding programme is undertaken before a new variety is named and released. The sequence of events leading up to the naming and releasing of a new variety is shown in figure 2 .

During my placement at East Malling, I carried out an experiment on Botrytis resistance in male sterile strawberry plants. I also worked on most aspects of strawberry micropropagation.



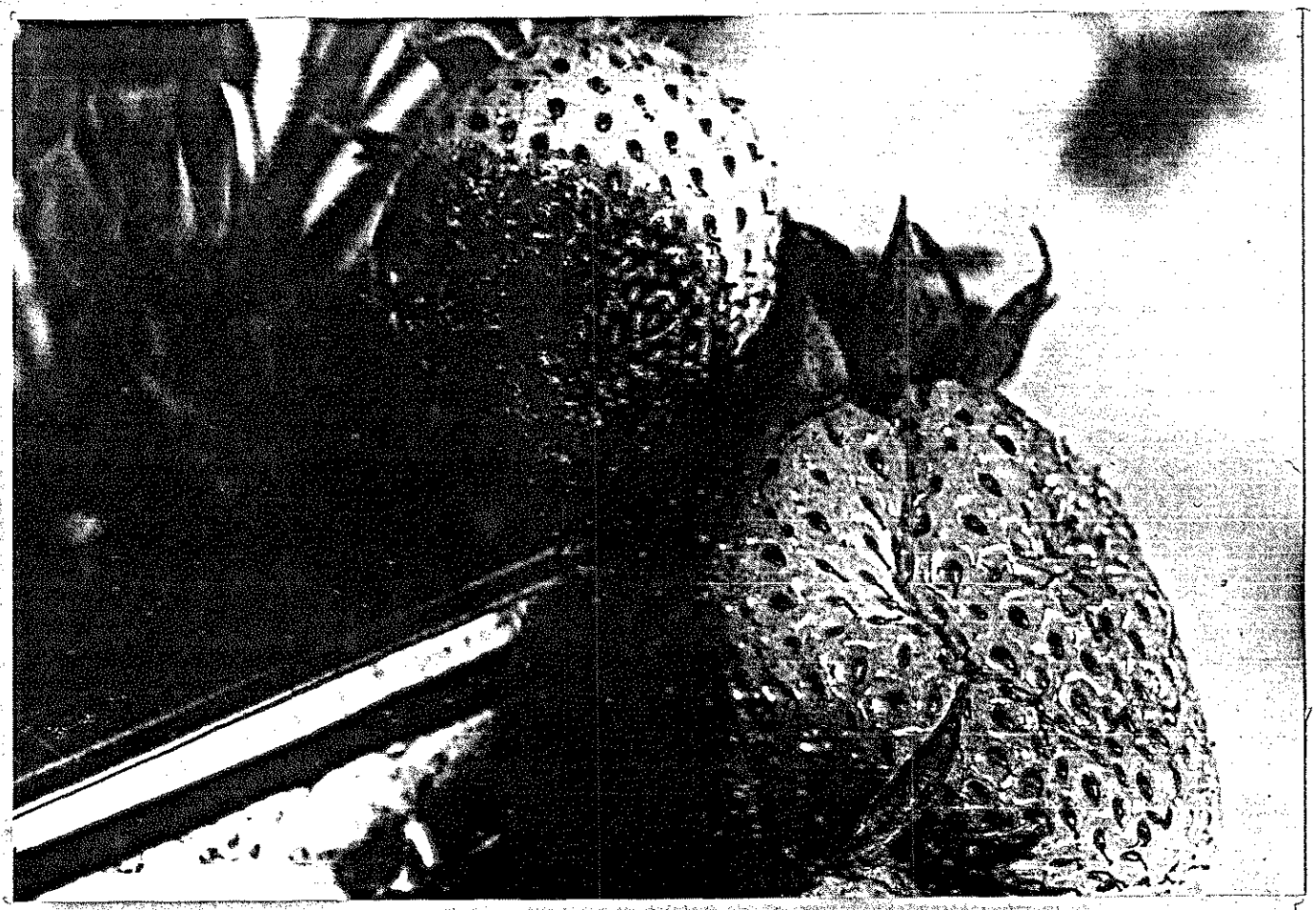
Different shapes of strawberry fruits

Figure 2.

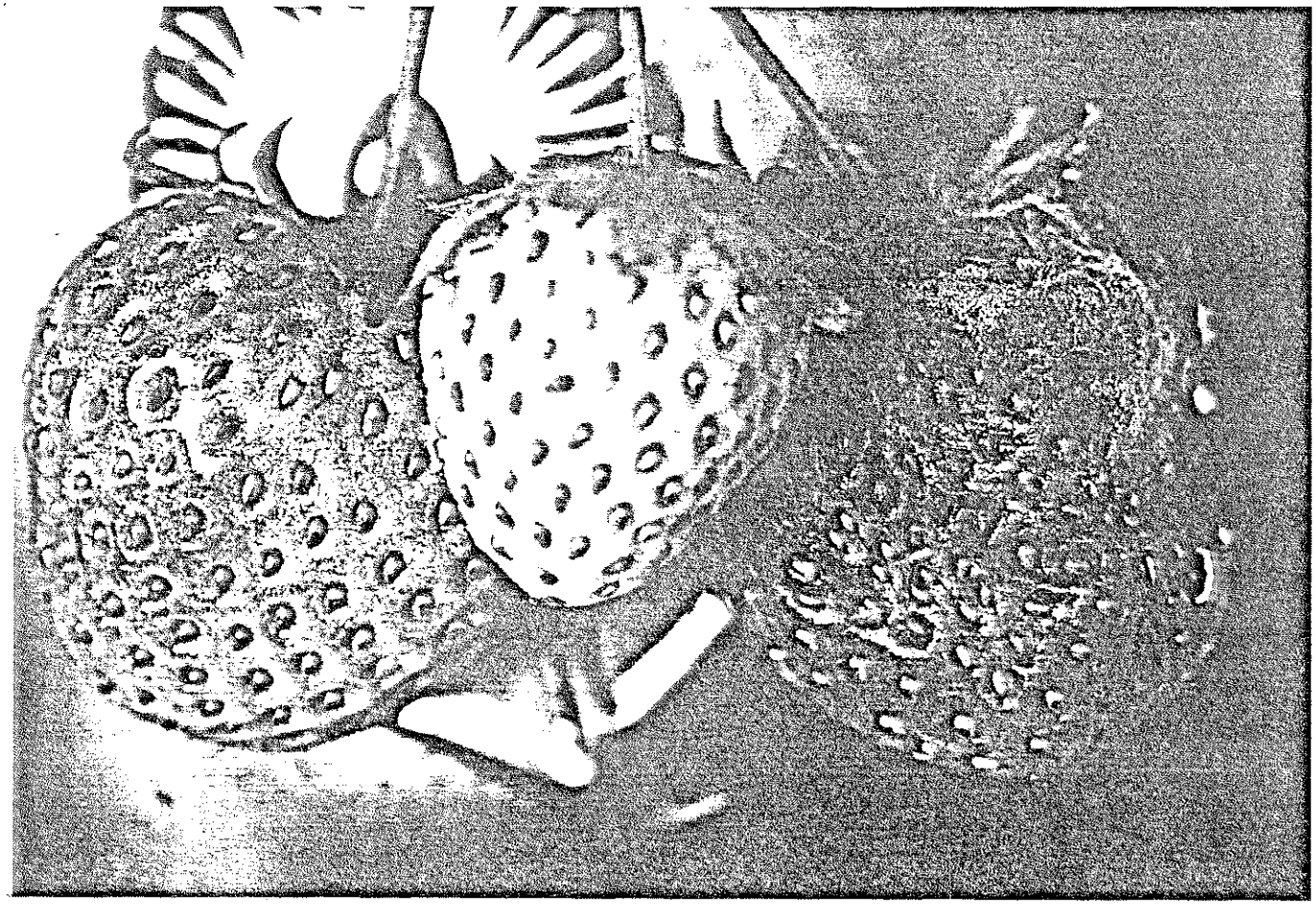
BREEDING PROGRAMME

YEAR	PROGRAMME	NO. SELECTED
YEAR ONE	CROSSING PROGRAMME	
YEAR TWO	SEEDLING POPULATION	15,000
	PRIMARY SELECTIONS	120
YEARS FOUR & FIVE	TRIALS AT EAST MALLING RESEARCH STATION	
	ADVANCED SELECTIONS	10
YEARS SEVEN AND EIGHT	TRIALS AT IHR, NFT, SCRI, SELECTED GROWERS & EHS'S	
	POTENTIAL VARIETIES	1 OR 2
YEARS TEN TO ELEVEN	MULTICENTRE TRIALS AT EHS'S AND GROWERS	
YEAR TWELVE	NAME NEW VARIETY	
	RELEASE TO GROWERS	

IHR - Institute of Horticultural Research; NFT - National Field Trials; SCRI - Scottish Crop Research Institute; EHS - Experimental Horticultural Station.

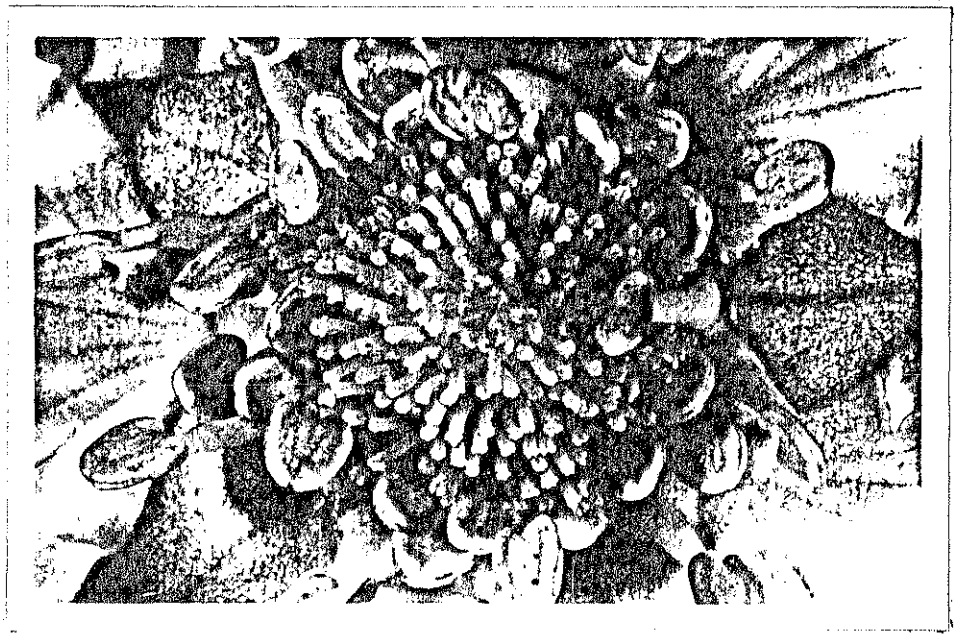


Botrytis cinerea on green fruit

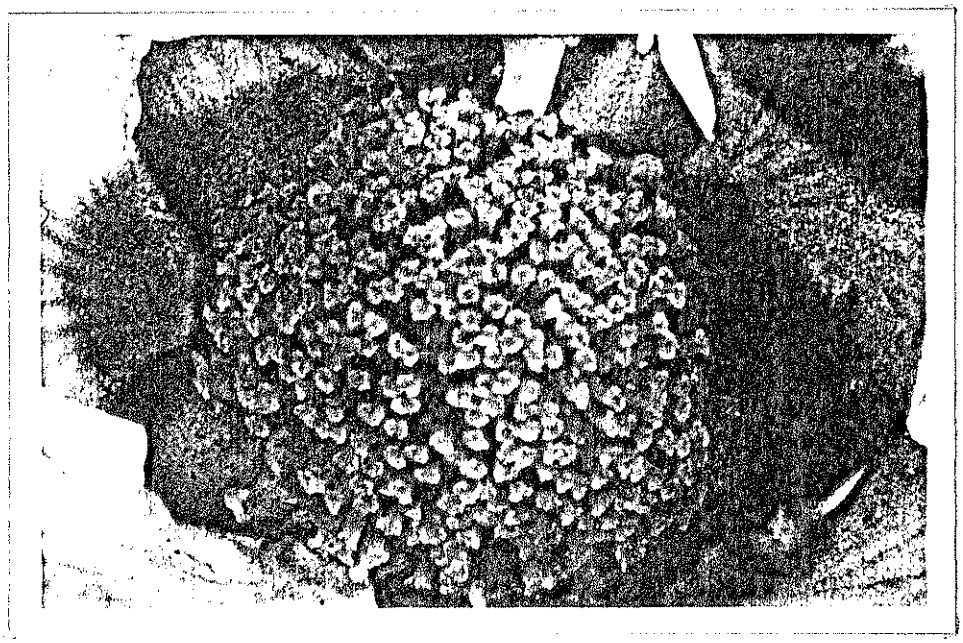


Botrytis cinerea on ripe fruit

FLOWER TYPES



Hermaphrodite



Pistillate

BOTRYTIS CINEREA INFECTION IN PISTILLATE AND HERMAPHRODITE STRAWBERRY FRUIT

Abstract

Two pistillate strawberry breeding lines at East Malling were observed to be showing some field resistance to grey mould caused by Botrytis cinerea. As infection by B.cinerea occurs at flowering time it was considered that the resistance may be connected with the absence of stamens on the flowers. An experiment was carried out in 1987 on two sets of flowers which had been removed from the plants (one pistillate and one hermaphrodite),the results showed a lower mean of infection in pistillate flowers as compared to hermaphrodite flowers. In 1988 further investigation of this hypothesis was done by innoculating the flowers in situ on the plants. Fifty-six plants were arranged in a glasshouse according to a randomised block design, and sprayed with a B.cinerea spore suspension four times over the flowering period.

Examination of the amount of fruit rot showed a slight increase in rotting on hermaphrodite as compared to pistillate plants, but this was not statistically significant. The overall level of fruit rotting was unexpectedly low.

Introduction

B.cinerea causes both green and ripe fruit to rot which can result in heavy crop losses. It is a facultative parasite occuring throughout the world and found in all strawberry growing regions.

The spores of B.cinerea are produced in large quantities and will only germinate under certain conditions. Dry spores only germinate where a film of water is present and only when it exists for some time ie. in the floral parts. Thus flowers can be infected from the bud stage onwards. Few infections occur from spore germination on the fruit, due to the lack of a water film. Most fruit infections are initiated by mycelium growing saprophytically in contiguous plant material or by water held between such materials and the fruit surface. The flower petals are thought to be a major source of innoculum as they may stick to the fruit and form a water film (Jarvis & Borecka 1968).

The mycelium may remain quiescent as a latent infection until the fruit ripens. No true degree of resistance has yet been shown in any cultivar, and the major mechanism of control is by the use of chemical sprays at flowering time.

Variation in susceptibility among cultivars is mainly attributed to firmness of berries and skin strength (Gooding 1976,Barritt 1980). Bristow et al, (1986) studied the disease development on different floral parts and concluded that the stamens may be more important than styles as a source of latent infection. They observed that in some cultivars the disease grew to the base of a few filaments and then entered the receptacle.

One wild strawberry species,Fragaria chiloensis, has both dioecious and hermaphrodite plants. When pistillate plants of F.chiloensis are crossed with hermaphrodite F.x.ananassa, the

progeny segregate 1:1 for the two flower types.

At IHR East Malling two pistillate breeding lines, each a third generation back cross to F.x.ananassa, showed signs of resistance to B.cinerea. (Simpson, 1988) These plants had a dense foliage, with flowers concealed amongst the leaves, a situation normally associated with a high B.cinerea infection. It was considered that the resistance seen may possibly have been related to the lack of stamens, which are known to be one route of entry of the disease into the receptacle (Bristow et al. 1986), and also the lack of pollen grains. Pollen is known to enhance B.cinerea conidia germination (Borecka & Millikan 1973 , Chou & Preece 1968).

Materials and Methods

Culturing B.cinerea

To perform the experiment a spore suspension of B.cinerea was required. Sporulating samples of the mould were taken from infected plants and brushed over potato sucrose agar , these were then incubated at 20°C in 16hrs of light per day .

POTATO SUCROSE AGAR (PSA)

Ingredients

Potato extract	500ml
Sucrose	20g
Agar	20g
Distilled water	500ml

Peel and dice 200g main crop potatoes and suspend in muslin in 500ml distilled water in a glass beaker, boil for 10 mins. Discard the potatoes and add water . Mix well . Add and dissolve sucrose and adjust the pH to 6.5 . Add agar and bring to the boil to dissolve . Dispense and autoclave .

Method

Four families were used, Fx86/39, Fx86/40, Fx86/41, and Fx86/42, these were the progeny of four cross pollinations using one pistillate parent and an hermaphrodite one.

These plants showed three types of fertility, no anthers (no viable pollen), vestigial anthers (no viable pollen), and fertile anthers. Seven sterile plants and seven hermaphrodite plants from each family were arranged in a randomised design and the experiment was replicated once using a different randomisation.

The plants were arranged in a glasshouse on benches covered with capillary matting.

Spore Suspension

A spore suspension of B.cinerea was used to infect the flowers. Spores were dislodged from the sporylating plates by flooding with sterile distilled water and agitating with a glass rod, the suspension was then filtered through a Milk Filter (Clare Disc) and then through a Whatman 113V filter paper.

The suspension was then examined and using a haemocytometer the number of spores per $1cm^3$ of suspension was estimated.

Calculation of spore count

Small square size = $1/16mm^2 = 0.0625mm^2$

Depth over central platform = 0.2mm

Therefore volume = $0.0125mm^3$

Number of small squares counted = 4 x 4 = 16

Therefore volume = $0.0125 \times 16 = 0.2mm^3$

In $1mm^3$ there are n x 1/0.2 spores

In $1mm^3$ there are

50 spores counted for 16 small squares

50 x 1/0.2 = 250 spores

Therefore :

In $1cm^3$ there are

250×10^3 spores = 2.5×10^5 spores in $1cm^3$

The plants were sprayed at regular intervals, after each spray they were covered with polythene sheeting for 24hrs to create humid conditions for spore germination. The number of

flowers sprayed was recorded.

Assessment

When the majority of the flowers had been sprayed, assessment began after four days, and continued on Mondays, Wednesdays and Fridays until the end of fruiting.

The number of infected fruit on the plants was recorded. Ripe fruit was removed from the plant and left for 24hrs at room temperature, and then the number of infected ripe fruit was recorded.

The data was then entered onto a computer for statistical analysis.

Results

Analysis of variance

SOURCE	d.f.	m.s.	v.r.
FAMILY	3	0.0407	0.35
SEX	1	0.0759	0.65
FAMILY BY SEX	3	0.0584	0.5
RESIDUAL	99	0.1174	

Table of means

FAMILY	39	40	41	42
MEAN	0.251	0.180	0.172	0.174

SEX OF FLOWER	FEMALE	HERMAPHRODITE
MEAN	0.168	0.220

Standard errors of differences of means

	FAMILY	SEX
s.e.d.	0.0916	0.0648

The variance within families is greater than the variance between families showing that there is no significant difference between families. The same is true for the sex of the flowers and for the interaction between families and sex.

The table of means for sex shows a trend towards hermaphrodite plants being more susceptible to the disease, but this is not statistically significant.

Conclusions and Discussion

The hypothesis being tested here was that resistance to B.cinerea could be connected with the absence of stamens, as they may be the principle route of entry of the disease into the receptacle.

The experiment investigated the effect this had on fruit rot. The results showed no significant difference between pistillate and hermaphrodite fruit, but there was some evidence showing an increased incidence of rotting in hermaphrodite fruit.

SEX OF FLOWER	FEMALE	HERMAPHRODITE
MEAN OF ROTTED FRUIT	0.168	0.220

The results were not conclusive although the trend of the data supported the results shown by the flower infection experiment in 1987.

There was a general lack of rotting fruit on all of the plants which implies that the experimental conditions, i.e. the microclimate, were incorrect for the development of B.cinerea. The experiment will be repeated next year, using a more humid environment and closer spacing to enable the disease to develop fully.

MICROPROPAGATION

INTRODUCTION

The techniques of micropropagation are used worldwide in numerous areas of plant science. At IHR East Malling, it is an integral part of the strawberry breeding programme.

When a large number of plants are required, using aseptic tissue culturing techniques allows them to be produced rapidly. Several million plants can be produced from one mother plant allowing propagation independent of season. A stock of all the different plant genotypes can be stored in a small area, and may be split to produce new plants when required. All the plants produced are disease and virus free thus providing a healthy stock.

Comparisons between strawberry plants produced from runners and by micropropagation have shown that the micropropagated plants produce more runners and are more prolific than nursery propagated plants (Scott Cameron et al 1985)

Everbearing plants are notoriously slow to propagate as they do not produce runners freely. Micropropagated plants appear to produce runners more freely in the field thus making propagation easier (Scott et al 1985)

MICROPROPAGATION OF STRAWBERRIES

Two types of terminal meristematic material can be used to develop new plants, the stem tips and root tips. For strawberry propagation the stem tips are used. Due to the production of runners a large number of tips can be taken from one plant and, if no runners are present, tips can be taken from the plant crowns.

The meristem is approximately 0.3 mm or less in size and is dissected from the tip. It is then placed on initiation medium, followed by transfer and lastly rooting medium.

1. The establishment of initial culture from freshly excised shoot apices (initiation).
2. The subsequent step of multiplication (transfer).
3. The final step of preparing the multiplying shoots for their transfer to soil (rooting).

Dissection of the apical meristem

Runner tips are taken from parent plants and placed in water. Using a laminar flow clean-air hood to prevent micro-organism contamination, the outer leaves are removed with forceps that have been immersed in 99 percent alcohol and flamed. Forceps are used to lessen the possibility of cutting the softer underlying tissues. Under high power the inner leaves are cut away with a sterile scalpel leaving the shoot apical meristem with 1-3 leaf primordia in the centre of the tip. The meristem is carefully dissected out and placed on the initiation medium in a glass tube. The mouth of a culture tube is flamed and the tube is capped and sealed with clingfilm.

The tubes are placed in a rack and incubated in a growth room with 16hrs light at 22^{o}C.

After 6-8 weeks the cultures can be placed onto the transfer medium in honey jars, the tissue can be split into several plantlets. These are incubated in the growth room and subcultured at six weekly intervals until the required number is present. They are then transfered to rooting media where they develop roots. After 6 weeks they can be placed in pots of vermiculite in a propagator in the growth room. The vents are opened after four days and the lid removed three days later. Then they can be planted into compost and grown on in a glasshouse.

The plants can be kept at the transfer stage and maintained in a cold store at $4^{o}C$, this is one method for long term storage of stock plants.

Media

The composition of the culture medium greatly influences the success of plant micropropagation. It must provide all of the inorganic salts usually found in soil i.e. salts of nitrogen, potassium, calcium, phosphorous, magnesium and sulphur and it must also contain small quantities of the trace elements i.e. salts of iron, manganese, zinc, boron, copper, molybdenum and cobalt. Plant tissue culture medium not only needs to provide these major macro and micro nutrients but also carbohydrates, usually sucrose, to replace carbon which the plant normally fixes from the atmostphere by photosynthesis. Other organic compounds needed are vitamins, amino acids and growth regulators.

Summary :

- macronutrients
- micronutrients
- vitamins
- amino acids and other nitrogen supplements

- sugar/s
- undefined supplements
- growth regulators
- buffers
- a solidifying agent (nearly always agar).

The growth regulators are very important components of the media and are the distinguishing components of the three different types of media used for strawberry micrpropagation.

Macronutrient salts

The balance of salts used in the three types of media are based upon the medium developed by Murashige and Skoog, 1962 (MS).

Compound	Mg/l	Compound	Mg/l
NH_4NO_3	1,650.0	H_3BO_3	6.2
KNO_3	1,900.0	$MnSO_4.H_2O$	11.4
$CaCl_2.2H_2O$	440.0	$ZnSO_4.7H_2O$	8.6
$MgSO_4.7H_2O$	370.0	KI	0.8
KH_2PO_4	170.0	$Na_2MoO_4.2H_2O$	0.25
NaFe.EDTA	36.7	$CuSO_4.5H_2O$	0.025
		$CoCl_2.6H_2O$	0.025

Medium pH

The pH of a medium must be such that it will not disrupt the function of plant cell membranes or the buffered pH of the cytoplasm. But the pH will also affect the gelling efficency of the agar, it will influence the uptake of medium ingredients and govern whether salts will remain in a soluble form.

The pH generally used on all of the media for strawberry culture is 5.6 and is adjusted just prior to autoclaving using NaOH or HCl.

Micronutrients

The essential micronutrient metals iron, manganese, zinc, boron, copper, cobalt and molybdenum, are components of plant cell proteins and are of metabolic and physiological importance. At least five of these elements are, for instance, necessary for chlorophyll synthesis and chlorophyll function. Iron is required for the formation of amino laevulinic acid and protoporphrinogen, and is a component of ferredoxin proteins which function as electron carriers in photosynthesis. Manganese is also necessary for the maintenance of chloroplast ultrastructure and the photosynthetic process. Molybdenum and zinc are needed, as deficent plants have a decreased chlorophyll content and poorly developed chloroplasts; while copper atoms occur in plastocyanin.

Chelating Agents

These are compounds where each molecule is capable of holding a metal ion with several chemical bonds, therefore forming a ring structure. Metals can be held in solution by a chelating agent under conditions where free ions would not be soluble. The most efficent is ethylenediamine tetraacetic acid (EDTA).

Vitamins

Vitamins are required to perform certain essential catalytic roles in metabolism. Intact plants synthesise their own vitamins but individual plant cells in culture are less able to produce them. The vitamins most frequently used are thiamine (B_1), nicotinic acid (niacin) and pyridoxin (B_6).

Myo-inositol is also used as a plant "vitamin". It has some

beneficial effects on plant growth and morphogenesis. The balance of vitamins used in the three types of media are based on Murashige and Skoogs (1962) vitamin mixture.

SUBSTANCE	Mg/l
Myo-inositol	100.0
Thiamin HCl	0.1
Nicotinic acid	0.5
Pyridoxin - HCl	0.5

Sugars

Plant tissue cultures are unable to supply enough carbohydrate, therefore it is necessary to incorporate an energy source into the media. Sucrose is the most frequently used.

Agar

Agar is used as a gelling agent for solid or semi-solid plant tissue culture. Its advantages are :

- with water it forms a gel at c.100^{0}C and solidifies at c. 45^{0}C.
  Therefore stable at incubation temperatures.
- not digested by plant enzymes
- does not react with media constituents.

Plant Growth Hormones

The hormones are the components that define the type of media, ie. initiation, transfer or rooting. The three used in these media are :

IBA = 3-indolebutyric acid

BAP = 6-benzylaminopurine

GA_3 = Gibberellic acid

AUXINS

Auxins are used to promote the growth of callus, cell suspensions or organs ie. meristems, and to regulate morphogenesis.

IBA is a synthetic auxin.

MW 203.24

CYTOKININS

Cytokinins are also used as important regulators of growth and morphogenesis in tissue culture.

BAP is a synthetic cytokinin.

MW 225.26

GIBBERELLINS

Gibberellins can influence growth and development in a variety of ways ie. increase stem length.

MW 346.38

The balance of these three hormones determines the type of plant growth.

Initiation media contains low concentrations of auxin and

cytokinins ie. 0.5 uM of IBA and 1.0 uM of BAP. This is to promote shoot growth on the meristems. The balance is very specific to strawberries. The cytokinin appears to be necessary for plant cell division and at high concentrations relative to auxin inhibits root formation.

The transfer media contains all three hormones, ie.

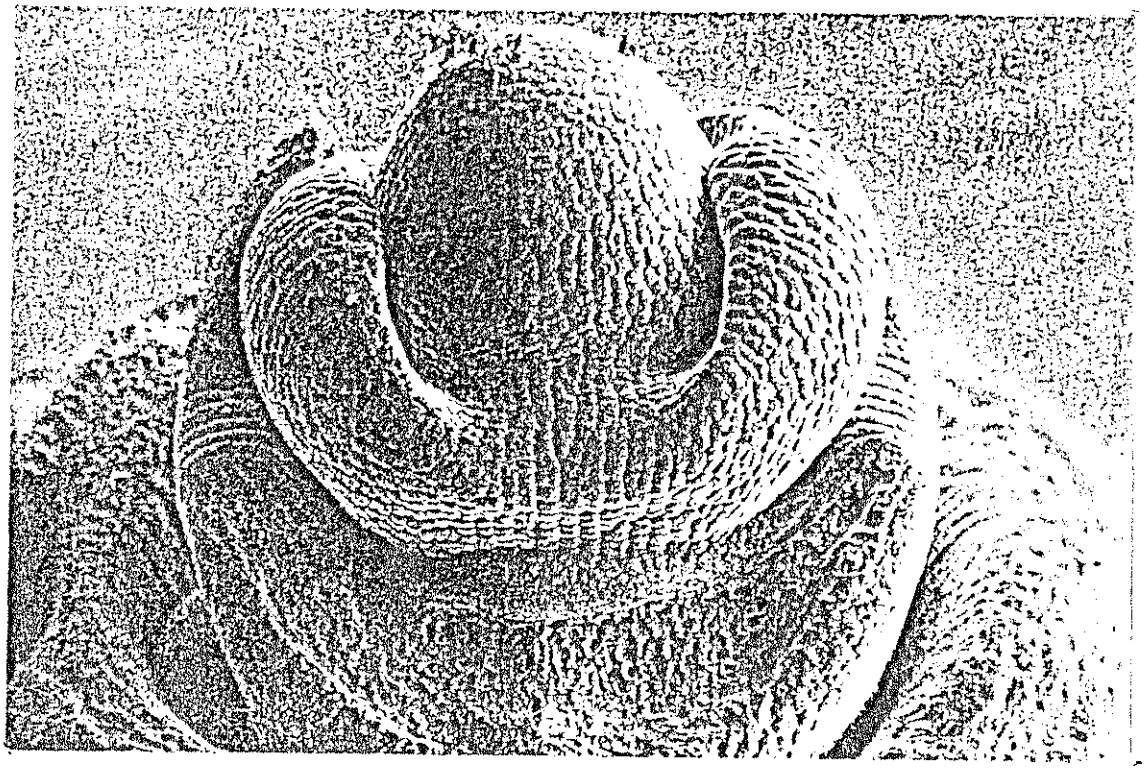
IBA - 1.0 uM BAP - 1.0 uM GA_3 - 0.3 uM

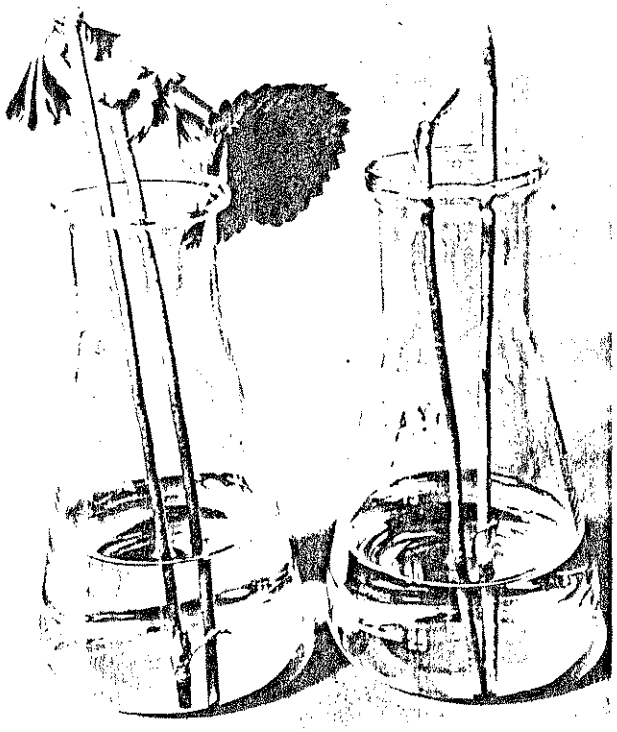
The transfer medium promotes shoot growth and cell division. The amount of auxin is increased as the explant is larger and requires higher hormone levels.

The rooting media contains a high IBA concentration to encourage root formation i.e. 2.0 uM.

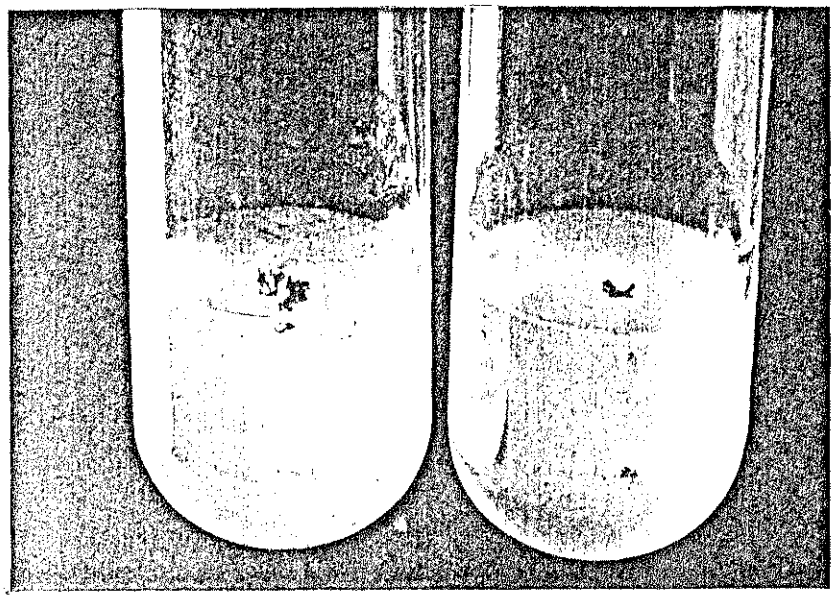
The balance of the three hormones in these recipes is entirely specific to strawberries.

APICAL MERISTEMS





Runner tips



Meristems in initiation medium

Plantlets in transfer medium

Plantlets in rooting medium

CONCLUSIONS TO PLACEMENT

I have enjoyed my time at East Malling IHR and would like to thank David Simpson and Judi Bell for making the last six months an interesting and rewarding placement. I have developed new skills in micropropagation and electrophoresis that I would like to develop further, I have also played a small part in the long and complex procedure of selecting and developing new strawberry varieties. I would like my next placment to specialise in the area of tissue culture.

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APPENDIX 1

Courses Attended

Introduction to the VAX computer course.

Introductory talk on basic statistical principles of the design of comparative experiments.

Half day at Fruit Focus 1988

APPENDIX 2

Tissue Culture Media

MS1

NH_4NO_3	33 g
KNO_3	38 g
KH_2PO_4	3.4 g
$CaCl_2.2H_2O$	8.8 g

- dissolved in 1 litre of distilled water.

MS2

$MgSO_4.7H_2O$	37 g

- dissolved in 1 litre of distilled water.

MS3

NaFe EDTA	3.67 g

- dissolved in 1 litre of distilled water.

MS4

H_3BO_4	3.1 g
$MnSO_4.4H_2O$	11.14 g
$ZnSO_4.7H_2O$	4.3 g
KI	0.4 g
$Na_2MoO_4.2H_2O$	0.125 g
$CuSO_4.5H_2O$	0.013 g
$CoCl_2.6H_2O$	0.013 g

- dissolve in 500 mls of distilled water.

MS5

Nicotinic acid	125 mg
Pyridoxine-HCl	125 mg
Thiamine-HC	25 mg

- dissolved in 250 mls of distilled water.

INOSITOL

5 g Myo-Inositol dissolved in 100 mls of distilled water.

BAP

40 mg BAP in 1 litre of distilled water.

GA_3

21.6 mg GA_3 in 250 mls of distilled water.
Dissolve in a few drops of 1M NaOH, make up to 250 mls with distilled water and alter the pH to approx. 5.

IBA

12.7 mg IBA in 250 mls of distilled water. (same method as for GA_3)

BASIC CONSTITUENTS

MS1	50 cm^3
MS2	10 cm^3
MS3	1 cm^3
MS4	1 cm^3
MS5	1 cm^3
INOSITOL	2 cm^3
SUCROSE	30 g

- DISSOLVED AND MADE UP TO 1 LITRE.

INITIATION MEDIUM

IBA 2.0 cm^3

BAP 5.6 cm^3

TRANSFER MEDIUM

IBA 4.0 cm^3

BAP 5.6 cm^3

GA_3 1.2 cm^3

ROOTING MEDIUM

IBA 8.0 cm^3

GA_3 1.2 cm^3

pH altered to 5.6 using a pH meter.

Add agar.

Heat in a microwave until agar dissolves (about 10 mins per litre).

Pour into tubes or jars and autoclave for 15 mins at 1 Bar pressure.