SOME FACTORS AFFECTING THE ESTABLISHMENT AND GROWTH OF BUD GRAFTS OF ROSES

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ABSTRACT

A brief review of abnormal growth of rose scion buds and the high proportion of buds which fail to produce normal growth in the production of rose plants by bud-grafting in New Zealand, introduces the subject.

The history of investigations, prior to 1968, into this problem is outlined. A description of normal growth patterns of shoots on roses, of axillary buds and of production methods in New Zealand is followed by a detailed description of the abnormal teratomatous growth, known as proliferation, which is characteristic of a proportion of affected buds.

The proposal is put forward that this condition is caused by infection with an organism that initiates galling similar in appearance to crown gall and that subsequent symptoms develop consequent to such a tumorous transformation but not necessarily due to the continued presence of the causal organism. In this study no causal organism was successfully isolated. The claims of a number of possible other causal agents are examined including development of the graft union, chemical factors, non-transforming bacteria, mites, fungi and viruses. Reasons are advanced why none of these provide a satisfactory explanation in agreement with experimental and observational evidence. This evidence is discussed in relation to the etiology and development of the disease syndrome.

The evidence presented is strongly in support of

a tumorous transformation, initiated by a soil-borne pathogen, occurring at the proximal end of the original stock cutting at the time of its insertion in the ground. Root initiation may be restricted by the position of the gall and the first shoot growth at the top of the stock may show slight symptoms. The infected plant is predisposed to show subsequent symptoms but these are dependent on subsequent operations and the time and conditions when they are carried out. These symptoms are the development of excessive callus at the point of excision of the stock top and at the incision of the bud-graft, the production of teratomatous shoots, known as proliferation, by the scion bud or failure of the scion bud to grow despite a successful graft union. These symptoms may be reduced or prevented by successful normal growth of the scion. A comparison of the etiology and development of the syndrome of proliferation disease is made with the classical characteristics of the crown gall syndrome. Recent research publications on crown gall are reviewed to establish that recent findings are not contrary to the proposal that rose proliferation disease is caused in a manner directly analagous to crown gall. It is proposed that the evidence supports the assumption that a particular strain of Agrobacterium tumefaciens or some bacterium closely related to it is the etiological agent causing rose proliferation disease.

PREFACE

An investigation of this nature involving woody plants and a seasonal expression of symptoms tends to spread over several years. The failure to isolate a causal organism is perhaps a reflection of the difficulty of the task. The failure of other researchers to achieve that end is perhaps some consolation. The lack of a nicely "sewn up" cause and effect has led to the involvement of a wide range of disciplines. The broadness of the field covered and the vast amount of possibly relevant information has prevented the carrying of many aspects of the investigation to any great depth and the description has accordingly tended to be disjointed. Over two thousand references have been card indexed and assessed but limitations of space and time have prevented the inclusion of more than a couple of hundred of the more pertinent ones in this thesis.

I must express my thanks to Professor R. Thomas and the Botany Department at Massey University for their patience. In particular to V. Sarafis whose thought provoking comments were a constant source of inspiration. Thanks are also due to Dr. A. Rainbow for his co-operation with his own research work and results on the same site, to Drs. .R. Elliott, M. Dye, D. Dye, P. Fry and K. Hammett at PDD whose comments and the opportunity to view and assess some of their experiments was most helpful. Members of the Nursery Industry throughout New Zealand have always

willingly given me the opportunity to make observations and assessments on their crops and made the results of their stock trials available for analysis.

As the former President of the United States, T. Roosevelt once said "scientists are those who view one another's work with quarrelsome interest." The inclusion of anyone's name in these acknowledgments does not necessarily infer that they agree with, or condone any, of the expressions of opinion or interpretations contained in this thesis, they are entirely my own.

Last but not least my thanks to my wife and family for their patience and long-suffering understanding.

P.C. Gardner,

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INTRODUCTION:

At least since 1954 and probably earlier, many commercial rose plant producing nurseries in New Zealand have been faced with increased failures of bud-grafts, following the bud grafting operation and in the early stages of scion growth, as compared with a relatively low proportion of failures in earlier years. Some of the scion buds which start into growth and subsequently fail exhibit a striking teratomatous growth pattern of the "Witches Broom" type. A very high proportion of bud failures and losses of scion shoots occur in nurseries in association with these abnormal growths and the total losses may be up to 90%.

Despite considerable investigation by a number of workers no completely satisfactory explanation of the cause of these teratomata has been published to date.

Perhaps coloured by memories of the "good old days" older nurserymen maintain that 90 to 95% bud take and 80 to 90% saleable yield used to be normal in rose production. Since the onset of these problems, and no doubt there are several, yields of as low as 10% saleable bushes have been recorded with a usual yield in most nurseries of 50% $\pm$ 25%. Many nurseries with heavy losses have given up growing roses as an uneconomic crop.

Occurring over the same period and no doubt

associated with some of the nursery problems there has been an increasing incidence of decline of rose bushes after transplanting into their permanent position.

Due to the involvement of several different diseases, some of which may have symptoms in common, it is not surprising that there has been considerable confusion of terminology both in describing symptoms and in naming the diseases involved. In this paper the terms will be used in the following senses.

The symptoms which may occur due to various causes are defined as follows:-

Blow Out is not strictly a symptom or a disease but the mechanical breaking off of a scion in the vicinity of the graft union caused by wind pressure.

Proliferation is the symptom of multiple shooting from the scion bud with the leaf lamina either vestigial or reduced in size with a reduced number of leaflets giving a "witches broom" type teratomatous growth (Figs. 8 & 9).*

Pinch off refers to the symptoms originally described by Roberts (165)* under that name of which the salient characteristic is that, despite an apparent successful

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*Note: Numbers in brackets refer to the appropriate references in the bibliography. Capital letters in brackets refer to the appropriate appendices. Figure and table references are prefixed with "Fig." or "Table" as appropriate.

graft between the bud shield and the stock, the scion shoots fail due to a rupture of the shoots from the bud shield because of anatomical weakness at the base of the rapidly expanding shoot.

Graft failure is a failure to establish a union between the bud shield and the stock. This may be due to a number of causes such as poor budding technique, loose tying, drying out, presence of various pathogens at the graft interface and others.

Bud failure is a symptom which refers to those cases where a more or less successful union is established. between the stock and scion shield but the bud itself fails to grow while the shield may remain alive for some time.

Die back is a symptom which may arise due to a number of causes and merely refers to the basipetal dying back of a shoot from the tip.

Rosetting is used to describe the characteristic appearance of a single shoot in which the internode lengths are grossly reduced to give a tightly packed rosette of leaves. This condition is quite distinct from a superficially similar effect due to several shoots arising from the same node (Fig. 21).

Epinasty is used in its ordinary botanical sense for a down-bending of the petiole unless referred specifically to other organs and may be due to a number of causes.

Excessive Callus refers to tissue similar in macroscopic appearance to wound healing periderm but appearing in considerably greater volume than is normal for wound

healing in the species under consideration (Fig. 18).
Galling is used as a general term to refer to any large (3 to 50 mm or more in diameter) tissue growths usually more or less nodular in structure and which only have a narrow point of attachment to the plant. Galls may occur below ground in which case the tissues are relatively soft and white with exposed actively growing cells on the surface (Figs. 11 & 14), or they may occur above ground when they usually have a corky, brown or greenish surface and are generally harder in texture (Figs. 19 & 20). These differences appear to be due to microenvironmental conditions. A gall at ground level may exhibit below ground characteristics where it is immersed in the soil and above ground characteristics where it is exposed. The general term galls includes tumors.
Tumor is used in a more restricted sense than gall to apply to autonomous gall growths including genetical tumors and crown gall transformation tumors. Tumor-like would accordingly mean having the appearance of such tumors (Figs. 11 & 14).

Diseases which appear to be primarily caused by a single etiological factor are referred to as follows:-
Blast is used only to refer to the disease caused by Pseudomonas syringae van Hall.
Crown Gall is used only to refer to the disease, characterised by tumors and associated symptoms, caused by the crown gall bacterium, Agrobacterium tumefaciens (Sm. & Townsd.) Conn.

Proliferation disease is the disease which has its most characteristic expression in the development of proliferation symptoms but, as is described elsewhere in this paper, may be expressed by the symptoms of pinch off, bud failure, excessive callus or galling.
Verticillium Wilt is the disease caused by infection with Verticillium dahliae Kleb.
Rose Wilt Virus is used in the sense of Grieve (80, 81) and refers to the disease characterised by the symptoms described by him in 1931 and experimentally transmitted. This term is not used, unless indicated, in the sense of Fry & Hammett (68) as far as the range of symptoms are concerned, nor to refer to the full range of symptoms ascribed to it by Hammett (84) or others following these writers in more popular publications. As will be discussed in more detail later it appears that these workers successfully transmitted a graft transmissible disorder with similar symptoms to Rose Wilt Virus (RWV) but without justification attributed a wide range of other symptoms occurring in roses to the same disorder.

HISTORICAL:

In order to clarify the present position with respect to knowledge of rose production problems in New Zealand a brief historical sketch of observations, comments and investigations relating to those aspects which have caused particular confusion is outlined. Only points relating to low yields in nurseries and decline of mature bushes are included and any disorders with a clearly circumscribed range of symptoms and

causal agent are ignored as not being pertinent to this investigation.

Most of the nurserymen's observations and the research institution's investigations have been reported to and co-ordinated at the Nursery Stock Research and Extension Advisory Committee (NSREAC). Much of the historical information is drawn from this committee's minutes (153), reports made to it (139, 152, 159, 160) and the writer's own personal involvement with it.

The NSREAC is a government advisory committee administered by the Horticulture Division of the Department of Agriculture. Represented on it are all the main institutions and organisations concerned with research and advisory work relating to nursery production together with three representatives of the nursery industry.

The committee was first formed in 1954 and as problems in rose nurseries were then apparent this matter came immediately on its agenda. The term "dieback" was used at this time to collectively cover the problems. The committee's annual report for 1955 states:-

"Dieback in established rose plants has been traced to various causes. Two common causes are:-

1. Infection of the roots by crown gall bacteria, and

2. Infection of the plants by verticillium wilt."

Blast had already been recorded in 1951 on roses in New Zealand and no doubt due to conditions being

suitable for rapid multiplication of the causal bacterium it was a serious problem in spring 1956.

The suitability of stocks in use and their possible infection with virus was questioned. In 1956 trials with various varieties of stocks were laid down by Plant Diseases Division (PDD) Auckland. A collection of stocks from a number of different sources were tested at the Horticultural Research Centre (HRC) Levin from 1960 onwards.

In 1963 at the July meeting of the NSREAC, Salinger, the then secretary, brought forward the information on pinch off in the paper by Roberts (165) and also read correspondence relating to a similar condition in South Africa thought there to be a virus. At this stage the writer drew attention to the apparent correlation between multiple shooting of the scion and excess callus. The term proliferation was first used to loosely cover proliferation of the shoots and wound tissue.

In October the same year nurserymen's representatives stressed that as well as the problems in the nursery that dieback and death in the customers gardens the year after transplanting was becoming an increasing embarrassment. In this respect the performance of some varieties in certain colour ranges were particularly involved.

At the March meeting 1964, Boyce of HRC reported that no statistical difference had been found in the root stocks under trial at Levin but 3 of these had been empirically selected and sent to PDD Auckland for

indexing. He stated, referring to the problems experienced by nurserymen, "It was not at the time clear whether this was due to blast disease, blow out or proliferation, or more recently loss of bushes after planting out."

In March 1965 the case of a nursery in Hamilton was quoted where a 75% loss in the 1964 crop and a 60% loss in the 1965 crop had been experienced. South Island nurserymen made the observation that in many instances there was an apparently healthy grafted bud shield but the bud itself was dead. The writer drew attention to an apparent correlation between high sap flow at time of budding and increased incidence of proliferation when those plants budded at such a time produced scion growth in the spring. The matter of anatomy of the graft union was also raised.

A report by Hunter of PDD was received in July 1965 to the effect that of the three stocks selected at HRC all were free of verticillium wilt but two of them had shown line pattern virus symptoms on indicator plants. It was noted that investigations on the use of seedling rootstocks were under way. Gardner suggested that a number of factors relating to proliferation had much in common with crown gall which could cause abnormal growths and that the excessive tissue growth, occasional noting of galls, the association with high wound sap flow and its being associated with humid conditions at time of budding favourable to bacterial infection would seem to support this suggestion.

At the March 1966 meeting it was recorded that Rainbow of HRC had prepared a report on proliferation investigations and visits to properties (Details of this unpublished report will be summarised later, together with other unpublished reports by the writer).

In winter 1966 the first distribution of cuttings of the clone of Rosa multiflora designated 56/7 was made from HRC. This was the one clone of the three tested at PDD and found to be free of both verticillium wilt and rose viruses known at the time to occur in New Zealand.

A special meeting between research workers and nurserymen was convened at HRC Levin on 19th March 1967 to discuss rose production problems. Various unpublished reports (159, 160) were presented to this meeting and will be summarised later.

At the July meeting of the NSREAC reports indicating losses as high as 90% and also as low as to be insignificant were noted. The pathogen Leptosphaeria coniothyrium (Fckl.) Sacc., was reported as having been identified on some proliferated buds.

Gardner again raised the question of crown gall and suggested that the bacteriological angle should be investigated. The meeting was advised that Dr. D. Dye the bacteriologist at PDD "... had definitely decided that it was not caused by crown gall...". "PDD consider that viruses seem to be the most logical cause".

Gardner made the further comment that failure to isolate the crown gall bacterium from the bud union and excessive callus tissue could hardly be regarded as evidence that it was not the causal organism as the

presence of the bacterium is only necessary at time of wounding to initiate the disease and frequently workers have found it difficult to isolate the causal organism from older galls.

It was also proposed that an extensive field investigation on a number of selected nurseries over a 2 year period be initiated. Marcussen stressed that in his area (South Island) the problem occurs in October and November, when buds fail to grow away.

Summary of Observations and investigations prior to 1968:

As the present investigation was initiated at about this time a summary of knowledge of this problem in New Zealand would be pertinent particularly as most of the information to this point is contained in unpublished reports.

The first adequate description of the proliferation symptom is contained in an unpublished report by Rainbow (159). This was prepared from inspections of a number of nurseries towards the end of 1965. He records the following investigations.

Work carried out by PDD, Auckland.

1. Despite frequent isolations from bud unions of proliferation affected plants the callus tissue that often occurs there and at the top of the stock no pathogen has been consistently isolated.

2. The blast bacterium was isolated from some of the proliferated shoots that had died back. An experiment was carried out treating to avoid blast. The treatment controlled blast but there was just as much proliferation in treated plants as in the controls.

3. To test for virus some plants were found showing symptoms on which the stock also had grown away. These stock shoots were taken for cuttings and the resultant stock plants were budded with eyes from the same infected bush. Only one plant out of 216 so treated showed proliferation symptoms.

4. An experiment was carried out using either raffia or plastic tape for tying and these were each used tied either loosely or tightly. The results failed to indicate that these tying techniques affected the incidence of proliferation.

Rainbow reported the examination of a small number of stocks with callus growth for mites with negative results. Briefly his comments referring specifically to proliferation disease are as follows:-

1. Of recent appearance in New Zealand and building up on some properties to catastrophic proportions suggesting a pathological cause.

2. Incidence varies from year to year on the one property.

3. It affects both standard and bush roses.

4. Incidence varies tremendously from property to property in the same year.

5. There is some evidence that the incidence on any one property in any one year varies with such factors as the time of budding and the condition of the stock.

A further extensive report by Rainbow (160) on a series of experiments in 1966 showed clearly the time-course pattern of proliferation symptom expression, with some plants failing and some growing out of it and

becoming apparently normal. Neither the incidence of proliferation nor other losses were affected by the application of either potash or boron. The fungus Leptosphaeria coniothyrium was frequently isolated from proliferated plants with poor unions but it was thought unlikely to be the cause of proliferation.

Other experiments by Rainbow but not reported on at this stage include innoculating buds with L. coniothyrium which showed no conclusive evidence of this fungus causing proliferation. The budding of stock from affected plants with buds from plants which had shown symptoms, again with no apparent increase in proliferation compared with controls.

Pertinent points on proliferation and observations made in unpublished reports by the writer (74) over this period and not already contained in the reports above are as follows:-

1. Some scion varieties tend to show the condition more than others but this may be due to the ability of some stronger growing varieties to overcome it.

2, Budding eyes imported direct from the Northern Hemisphere in November show few proliferation symptoms when budded at this time. These plants however are normally headed back in early January to force the imported varieties into growth. Budding at this time and heading back in early January using local budwood also shows practically no proliferation symptoms.

3. As soon as recently imported varieties are budded at the normal time of the year and treated as for normal production they show the same incidence of

proliferation as older varieties.

4. Active stock growth and heavy sap flow at budding time seems to increase subsequent expression of the symptoms.

5. There generally appears to be less incidence in drier localities with less lush growing conditions.

6. The use of watertight tying materials at times of high sap flow thereby retaining excessive sap at the point of budding tends to encourage subsequent symptom expression.

7. The plants which show proliferation symptoms with excessive callus at the point of budding usually also show excessive callus at the point where the stock top has been removed several months later and often 2 or 3 inches above, with no symptoms between these points. This indicates that the tendency to form excessive callus is systemic and dependent on wounding.

Note:- all the comments and observations in the above reports relate to proliferation symptoms as Rose Wilt Virus symptoms sensu Grieve (80, 81) were not recognised as a problem in New Zealand at this point of time.

DESCRIPTIVE:

Normal growth patterns of rose cultivars.

The shoots of a bush rose cultivar formed in any one season are all determinate and no terminal winter buds are formed. All shoots which develop following the dormant period arise from lateral buds.

An elongating shoot axis makes rapid growth and becomes determinate either by the formation of flower

buds at its apex or the apical meristem ceases activity and often becomes displaced. Once such a growth cycle is completed any subsequent growth even in the same season will only arise from a lateral axillary bud.

On an established plant the growth of a shoot arising from any one lateral bud will follow one of either two or three patterns depending on the characteristics of the cultivar or species.

Which of these growth patterns is followed is determined largely but not entirely by the relative position of the bud on the plant.

The first spring growth commences with buds near the top of the plant. This will be from shoot buds with larger, thinner, more loosely clasping bud scales than lower on the shoot, which had formed in the axils of modified transitional leaves in the region immediately below the previous seasons's flowers. These buds only elongate weakly to form short shoots 5 to 20 cms. often without fully developed leaves and terminating in small often malformed flowers.

Horticulturally it is normal practice to winter prune to remove all parts of the plant carrying this type of growth bud and to make the excision approximately 5 mm above a node in the position which normally next starts into growth. This is in the central third of a previous seasons shoot and is the region of a shoot which bears fully developed leaves with the maximum number of leaflets typical of the variety and in which the axillary buds attain the

greatest size and development (Figs. 2 & 3).

Growth from these buds is stronger and produces the type of shoot and bloom which is horticulturally desirable. These also are the axillary buds which are used for the bud-grafting shields in the propagation of further plants. The shoots which arise from these buds, describing from the proximal end, have at the base the 2 lateral secondary buds which were in the axils of the first pair of scales. Closely above these are the scars left by the remainder of the dehiscent bud scales. Although quiescent bud primordia are present in the axils of the bud scales these show little development on the normal shoot. At progressively increasing internodal distances up the shoot are the transitional leaves ranging from little more than stipules to almost fully developed leaves with one pair of leaflets less than normal. These transitional leaves frequently abscise by the time the shoot is fully developed. They bear in their axils differentiated lateral buds which are much smaller, project less from the stem and are less prominent than those in the axils of fully developed leaves.

Distal to the basal transitional leaves and median on the shoot are the fully developed leaves with the full number of leaflets characteristic of the plant. The axillary buds subtended by these leaves show the maximum development and are described later in detail.

This type of shoot normally terminates in either a flower or an inflorescence and distally from the

fully developed leaves there is a transition series reducing in size down to floral bracts. This floral transition series subtends axillary buds which are smaller and somewhat looser in the arrangement of the bud scales with a lesser number of true bud scales and often some leaf development even on the outer cataphylls.

After the main flush of spring flowering, which occurs during November, a third type of shoot arises from low on the plant and grows quickly to equal or over top the rest of the plant. Horticulturally these shoots are known as "water shoots". Frequently these shoots arise from dormant buds on wood more than 2 or 3 years old. These buds are frequently not superficially apparent prior to commencing growth as they may be completely covered by the periderm. Also they can arise from the previous season's growth low down on the plant in which case the bud may not have been obscured by periderm.

Basal shoots are vigorous and quick growing of large diameter with a proportionally large pith. The lateral axillary buds on these shoots are less prominent and often sunken. The basal shoots bear a large candelabra shaped compound panicle of flowers even on cultivars which bear a solitary terminal flower on the November flowering shoots. Shoots arising concurrently with the basal growth during summer or autumn may bear only solitary terminal flowers if they arise high on the plant. Growth intermediate in type between these basal shoots and

normal flowering shoots may occur from intermediate positions on the plant.

This generalised growth pattern shows a wide range of modification in the many cultivars and horticultural hybrids. The basic underlying pattern is apparent although hybrid cultivars have been selected for an increase, modification or suppression of the various growth features.

The highly specialised commonly grown fanciers roses are of multispecific origin and due to intensive interbreeding it is now generally difficult to closely identify the cultivars with the original specific types. More or less close relationship with the main lines of origin enable some horticultural collective groups to be recognised although continued cross-hybridising has produced a linking series of intermediate forms.

The generalised growth pattern outlined above is directly applicable to typical cultivars.

The so called climbing cultivars differ in the basal shoots, which are more or less analagous to the basal shoots of bush cultivars which produce in autumn large compound panicles of flowers, except that on climbers these shoots usually do not produce flowers at all during their growth but form long (2 to 4 metres), more or less flexuous, canes which cease growth without forming a terminal bud. The lateral buds of these shoots produce shorter (15 to 40 cm) flowering shoots the following spring. This condition can occur either due to the characteristic being determined genetically by breeding or by a chance bud

mutation occurring on a bush cultivar and producing a climbing shoot. Such mutations may revert to the bush form.

R. multiflora which is used as bud grafting stock in New Zealand has a similar habit of growth to the climbers outlined above.

Axillary buds:

The bud shields used for grafting are inactive axillary buds on woody shoots of the current season's growth. These are normally chosen in the case of bush rose cultivars from normal flowering shoots on which the apical flowering has finished but the hip has not yet matured. The more fully developed buds towards the centre of the shoot are used (Figs. 2, 3 & 22).

A typical bud of this type appears macroscopically as a pointed dome of cataphylls situated immediately above the point of attachment of the leaf or the leaf scar (Fig. 3). Surrounding the bud is a paler area. The typical internodal stem at this stage of development prior to the formation of periderm has an intact epidermis with longditudinal rows of stomata beneath which are longditudinal bands of loosely packed parenchymatous cortical cells containing numerous chloroplasts. Between these photosynthetic bands are supporting bands of collenchymatous tissue. In the paler area surrounding the bud there are no stomata and the chlorophyllous parenchyma forms a continuous layer but separated from the epidermis by a layer 3 to 4 cells thick of collenchyma. This submerged chlorophyll layer does not extend right

to the base of the cataphylls.

Extending from below the stomatal bands laterally adjacent to the bud through to the bud traces is an aerenchymatous area where the cortical cells are widely separated and form filamentous chains up to 6 cells or more long traversing the air space (Fig. 7). It would appear that this area serves to aerate the deeper tissue in the vicinity of the bud where no stomata are present in the epidermis. The function of such aeration is not apparent.

The vasculation of the subtending leaf and of the axillary bud is of a simple pattern with a single leaf trace and a stipular trace to each of the two stipules. The bud traces are comprised of two vascular strands arising one each side of the leaf gap and arching over to just below the apical meristem. The first two outer cataphylls of the bud are opposite and placed laterally at right angles to the subtending leaf. A small secondary bud is located in each of the axils of the first two cataphylls (Fig. 4). Where the primary bud traces pass below the lateral secondary buds they bulge outwards towards the secondary bud with a leaf trace leading to the subtending scale and a leaf and bud gap with lateral bud traces to the secondary bud.

The second pair of cataphylls are sub-opposite and are approximately at right angles to the first pair. Thereafter the phyllotaxy of the cataphylls and leaf primordia closely follow the $\frac{3}{8}$ divergence pattern of a mature shoot. The fully mature dormant

axillary buds have 6 to 10, usually 8 bud scales which enclose up to 8 leaf primordia which show some development of primordial processes that will ultimately develop into leaflets (Fig. 5). Surrounding the apical meristem of the axillary bud (Fig. 6) are 3 to 4 leaf primordias P_1, P_2, P_3, P_4 using the terminology of M. & R. Snow (1931) which show no differentiation of primordial leaflets. P_1 is a ridge at the side of the apical meristem and P_2, P_3, P_4 are progressively larger. Apart from the two lateral secondary buds differentiated in the axils of the first to cataphylls there is no differentiation of secondary buds at this stage although quiescent primordia with dense cytoplasm occur in the axils of all the cataphylls.

Formation of shoots from axillary buds.

Growth may be initiated in an axillary bud at any time during the growing season by cutting off the main axis at the internode above the bud. The primary bud meristem becomes active and elongation of the bud axis commences. The bud scales are sloughed off and the undeveloped leaves within the bud expand to form a transitional series from the proximal which is little more than a stipule with a vestigial lamina through increasing larger lamina with less than the mature number of leaflets to the normal leaf size and shape.

The secondary buds which were situated in the axils of the first pair of bud scales do not normally start into growth unless the elongating axis of the primary bud is checked in its growth, excised or

becomes determinate by the formation of flower buds at its apex. Even then lateral buds in the axils of fully developed leaves on the new shoot axis preferentially start into growth unless there is an excision of the shoot below this point, in which case secondary buds which were present in the axils of the first pair of bud scales swell and one or both form shoots. If these secondary buds have also been excised or damaged to prevent growth quiescent partly differentiated bud primordia which have remained dormant in the axils of the third and subsequent cataphylls may develop into buds and shoots.

Propagation methods for roses in New Zealand.

Briefly the usual method of production of plants of rose cultivars in New Zealand is as follows:-

Woody cuttings of the current season's growth are taken in early winter from strong canes 5 mm to 15 mm in diameter of various clones of Rosa multiflora Thunb. which have been selected for their horticulturally desirable characteristics such as ease of rooting, thornlessness etc. These canes are cut into 15 to 30 cm lengths and all the dormant axillary buds removed except for two at the distal end of the cutting. The dormant cuttings are inserted 6 to 10 cm into the ground in the open field, either immediately they are made or after they have been allowed to form callus on the wounds under moist conditions. With the onset of spring growth adventitous roots are initiated and grow at the proximal end of the cutting principally from the nodes and to a lesser extent from

the internodes. The two distal buds which are left intact, shoot and grow during the spring and summer to form a bushy head to the stock plant (Fig. 13).

The bud-grafting operation is carried out during the period November to March after the manner as described by Garner (1947 pp. 99 - 104) under the heading "Shield budding", and by Hartmann & Kester (1968 pp. 461 - 466) under the heading "T-Budding". The buds are inserted on the stem which was the original stock cutting as close to ground level as convenient and well below the top of the original cutting where the two most distal buds were left to form the growing top of the stock bush. Various minor modifications of technique are employed by different operators and of recent years various plastic strips, rubber patches and clip on ties have been used for binding the bud in place.

The establishment of a healthy bud graft union closely follows the pattern described by Buck (39, 40) and by Soraver (180). Variations from this basic pattern of bud graft union establishment will be discussed later.

After budding the plant is left with the stock top intact through the autumn and winter until early spring approximately 15 months after making the stock cutting. During this period the grafted bud usually remains inactive although some of the grafted buds may break into growth during the late summer and early autumn immediately after grafting.

In early spring the stock top is cut off above the grafted bud by a single cut through the stem that

was the original stock cutting. This removes the two distal nodes at which growth buds were originally left and all the top growth arising from them. As Rosa multiflora under field conditions does not form adventitous buds and the remaining part of the stem has had all the nodal buds removed from it the only point at which shoots can arise is from the grafted bud.

This should then break into vigorous growth and produce a fully grown rose bush by the following winter which is 2 years after the original stock cutting was made.

It is in the first few weeks following heading back of the stock tops that the failures and teratomatous growths become apparent.

No papers have been published to date adequately describing the condition. Many investigators have used the descriptive terminology in different senses, often combining several symptoms of probably different disorders under the one name.

Proliferation:

The term proliferation was first used by the writer in 1963 descriptively for the symptoms and subsequently as a name for the condition now widely known under that name.

The term proliferation was used as it was loosely applicable to both the wound tissue proliferation and to the production of multiple shoots from a rose scion-bud.

Subsequent authors have used this term to apply even more loosely to shoots which show epinasty and

distorted growth.

As used in this paper the term proliferation is restricted to the production of multiple shoots giving a teratomatous, "witches broom" type growth with prominent stipules and leaf lamina reduced or absent, (Figs. 8 & 9) (Ref. 68: Figs. 1 & 2). Epinasty may or may not be present and is not necessarily a symptom of this condition. Excessive callus or galling is generally present but is not necessarily apparent and is considered, as will be shown to be, another symptom of the conditions which give rise to proliferation.

The term proliferation disease of roses is proposed for the disorder which may be expressed by proliferation symptoms of the shoots arising from grafted buds in the asexual propagation of roses.

Proliferation disease may also be expressed by bud failure in those cases where the bud graft has formed a union of sorts but subsequently fails to produce shoots at all. Again excessive callus is usually present but may not necessarily be obvious.

This does not necessarily infer that any one of those symptoms on its own can only be caused by proliferation disease. Graft failure distinct from bud failure can occur due to a number of other causes such as faulty technique and various pathogens or directly as a result of galling at the graft union. Galling and no doubt excessive callus can occur also due to other causes.

Illustrations of typical proliferation symptoms

have already been published (Ref. 68 : Figs. 1 & 2), (Ref. 75 : Fig. 6) as have extreme cases of graft failure associated with galling (Ref. 75 : Figs. 7 & 8). The proliferation symptom may however vary widely in the extent of its expression but is always distinct from the condition transmitted by Fry & Hammett (68) which is typically expressed by down curling, epinastic leaflets on leaves with the full number of leaflets.

Those buds which produce proliferated shoots may do little more than produce a small cluster of shoots with typical symptoms but not more than 5 mm long before dying or the shoots may be anything up to 40 cms or more in length but still with fully developed stipules and leaves greatly reduced or absent.

The lateral buds on shoots showing proliferation symptoms may grow to form lateral shoots showing the same stipule and leaf symptoms or on occasions the lateral shoot may develop leaves with three normal sized leaflets but fully normal shoots are never produced arising from a proliferated shoot. (See Table I for comparison with normal shoot). On the other hand a bud may produce a number of typically proliferated shoots and then give rise, close to the bud union, to an apparently completely normal shoot or shoots which usually develop into a normal rose bush. When this occurs the plant does not at any stage revert to show proliferation symptoms nor do budding eyes taken from such a plant show any increased incidence of proliferation even when budded on stock plants grown from stock cuttings taken from plants

Table I:

Comparison of a typical proliferated shoot with a normal shoot (Nodes and appendages described in sequence from proximal end)

Normal shoot	Proliferated shoot
Lowest 2 to 8 nodes closely packed at base of shoot. 2 to 8 scales originating from original bud scales. Lower (outer) more or less deltoid. Upper (inner) progressively becoming ovoid to strap shaped.	As for normal shoot
Next 2 to 4 nodes with successively longer internodes (10 to 60 mm). The 2 to 4 stipules with vestigial leaves originating from the partly differentiated bud cataphylls.	As for normal shoot
1 to 2 stipules with lamina present but somewhat reduced in size and leaflet number. Probably formed from younger leaf primordia in bud.	1 to 2 stipules with vestigial leaves.
1 to many (depending on length of shoot) normal fully developed leaves with the full mature number of leaflets for the clone.	1 to many stipules with vestigial leaves or occasionally leaves reduced in size and leaflet number.
4 to 7 leaves with normal size leaflets. Leaflets reduced in number usually to 3. Uppermost frequently reduced to 1 leaflet. Upper 2 or 3 leaves often with very short internodes forming an involucral whorl below a terminal flower bud.	Some shoots die at tip. Others have stipules or reduced leaves as on main axis below. Axillary buds may form short shoots with less reduced leaves. These lateral shoots arise from buds which do not have true bud scales. A terminal flower bud may be formed.

which had shown proliferation, (152, 153, 161).

The impression gained from observation of the expression of symptoms in their various forms is that abnormal tissue growth both behind the scion and primarily within the scion itself interferes with the establishment of normal vascular continuity. This however is more than just a mechanical interference. In order to account for the gross morphological changes apparent in the proliferated shoots it would appear that the production of growth factors or interuption to the movement of growth factors or morphological controls probably occurs.

The first formed shoots elongate and then quickly cease growth sometimes shrivelling at the tips and becoming blackened and dry from the tip towards the base of the shoot (Ref. 68 : Fig. 2). After cessation of growth of the first shoots further shooting occurs either from the basal buds that were subtended by the cataphylls of the original bud or as proliferated shooting progresses from basal buds on the first proliferated shoots. The position and type of buds which give rise to these shoots is perhaps significant in view of the similarity of appearance between the expanded stipules with vestigial leaves on proliferated shoots and the very similar appearance of the inner cataphylls after they have expanded on a normal shoot, (see Table I) (compare Figs. 2 & 8).

It is a noticeable characteristic of severely proliferated shoots that they tend to be easily broken off at the base of the shoot between the shoot and

the original bud shield or growth from it (Ref. 68 : Fig. 3).

At this point there is a very noticeable constriction of the xylem cylinder.

The broken surface does not give the impression of long fibres, tracheids and vessels as in normal xylem tissue in normal scion shoots. It is virtually impossible to obtain a clean break at this point with a normal shoot and such a break as can be obtained has the normal characteristics of xylem tissue.

Microscopic examination of normal and proliferated shoots confirms this impression. An examination of longditudinal and transverse sections of the base of normal shoots as they arise from the grafted bud showed normal xylem characteristics for the rose. A similar examination of proliferated shoots at the point of breaking showed that the xylem cylinder was composed of pitted tracheary elements almost cubical in shape being very little longer than wide. The pits appear similar to those found in vessels and tracheids in rose xylem. The tissue containing these short cells was essentially homogeneous but becomes mixed with more normal elongated xylem elements 1 to 2 mm above and below the point at which the break occurs.

The proliferated shoots themselves have a very large area of pith (up to 90% of the diameter of the shoot) with relatively little vascular tissue and a thin layer of cortical tissues as compared with normal shoots. The large pith of proliferated shoots is

constricted at the base of the shoot and becomes little more than a thin trace. On proliferated shoots the xylem cylinder and cortical tissues are often constricted just below this point to as little as 10% of the diameter of the stem above. This constricted zone is where the short xylem elements occur and the proliferated shoot is easily broken off. In normal shoots the pith in this position is constricted in a similar manner but the xylem cylinder has approximately the same outside diameter as in the shoot above and the stem from the cambium to the centre is almost solid xylem. Accordingly in normal shoots this is the strongest point and almost impossible to break although the graft union itself may still be relatively weak and breakage can occur at that point. The graft union itself may also be weak in the case of scions with proliferated shoots but frequently no weaker than a normal union of equivalent age.

The condition known as 'pinch off' described by Roberts (165) and occurring in New Zealand in areas together with proliferation is similar morphologically to a single proliferated shoot and is probably another expression of the same condition but the first and only shoot fails or breaks off at the constriction before multiple shooting occurs.

As already mentioned a bud which has thrown a number of proliferated shoots may throw an apparently normal shoot and then develop as a normal plant. When this occurs the normal shoot always arises directly from the original scion and never from a

proliferated shoot. Usually it arises to one side of the proliferated growths and all normal shoots arising thus always have a normal vascular supply without a constriction in the xylem. The vascular connection between the normal shoot and the stock becomes woody in the same manner as a normal union.

At least in the early stages of proliferated shoot growth the tissue which develops between the base of the shoots and the actual graft union is relatively spongy. This area often becomes considerably enlarged with internal tissue growth and develops a rough corky surface with nodules and fissures similar in appearance to above ground galls.

Microscopic examination of these tissues shows a relatively disorganised mass of cells, parenchymatous at first but becoming more or less pitted as the growth matures. Multicellular strands of relatively short tracheary elements with thickened walls and heavily pitted appear to be scattered through the ground tissue. These strands appear to follow relatively random courses. Some make contact with the xylem of the stock and connect with the proliferating shoots others run into the callus nodules. Serial sections show that others are merely groups of cells with no vascular connection and occur as isolated islands of tracheary elements in the ground tissue.

With older proliferated scions secondary growth can be observed around the strands and the isolated islands of tracheary elements develop into amphivasal nodules. Those proliferated scions which produce a

normal shoot have extensive secondary growth forming a strong vascular connection between the shoot and the stock.

It has been suggested (74) that the successive growth and failure of proliferated shoots is caused by sufficient primarily differentiated vascular connections being established in the disorganised area to initiate growth of a shoot but consequent on the increased activity of shoot growth increased activity in the disorganised area tends to disrupt rather than establish adequate vascular connections to support the growing shoot. The shoot fails and the cycle is repeated with other basal buds until there is either complete failure or a shoot establishes a normal vascular connection usually to one side of the disorganised area.

The early stages of tissue development in this area is microscopically very similar to the tissues found in older callus overgrowths, and to a lesser extent galls, at ground level and above, there being similar scattered strands and islands of short thickened, pitted, tracheary elements scattered through this tissue and limited secondary growth may also be apparent. Many of the strands in these cases also run from the stock xylem to the surface nodules. The rough corky nodulated swellings which tend to occur at the base of the shoots on heavily galled stock cuttings also show very pronounced vascular strands running to the nodules (Fig. 12). Below ground galls

may also show similar development but to a lesser extent. Similarities between these findings and other galled and teratomatous growths is discussed in the main body of the text.

THESIS:

As already mentioned it had been suggested by the writer on numerous occasions that Agrobacterium tumefaciens (Sm. & Townsd.) Conn. or some other free-living organism causing a similar galling was the agent responsible for initiating the condition which led to proliferation although the causal organism may not necessarily be present at the time of development of the proliferation symptom. It was for the purpose of examining this proposal and the possibilities of alternative causes that this investigation was initiated.

Most New Zealand workers in the field were strongly opposed to any suggestion that an Agrobacterium might be the etiological agent (153) and were strongly of the opinion that the condition was caused by a virus. Nevertheless it was felt by the writer that the pattern of incidence in the field and the lack of evidence of transmission by grafting supported the assumption that proliferation was caused by a free-living organism which infected at some particular state in the development of the plant. The observed correlation of galling and excessive callus with wounding at various stages and the appearance of the teratomatous growth being similar to that known to occur with crown gall seemed to indicate an Agrobacterium.

Nevertheless other possibilities could not be discounted so a broad approach was made to the whole subject.

Wound healing and Graft unions:

In order to define and recognise the abnormal it is necessary to first become thoroughly familiar with the normal. Microscopic examinations of early stages of wound healing on R. multiflora revealed a general pattern in conformity with major treatises on the subject (21, 22, 180). Damaged cambium did not generally continue to be functional as found by other workers on R. multiflora (39, 40) Malus (171, 193), Prunus (64) and other plants (37, 47, 61, 151, 176, 179, 180). It was noted that on large established bushes of R. multiflora a careful, clean, lifting of a bark flap gave in ten days a wound healing response similar to the generally accepted pattern (39, 151) with the vascular rays making a major or sole contribution to callus formation (Fig. 23). On the other hand any deviation from this pattern such as scoring of the exposed xylem surface, innoculation with various bacterial cultures or carrying out the operation at the normal budding point of stock, which is close to the ground and difficult to keep free of contamination, resulted in a number of different types of response with callus arising from various sources. Any living tissue outside of the mature xylem cylinder in those wounds which were probably not sterile appeared capable of giving rise to callus. This is contrary to the findings of most workers but is in agreement with Sass (171) who also reported callus formed from any living tissue outside of the xylem cylinder in apples. This is of particular interest as he was

studying the formation of callus knots and overgrowths in the union of apple grafts. The "poor" trees which showed these overgrowths only yielded crown gall bacteria in 1% of those examined and he came to the conclusion that crown gall was not a major factor in causing overgrowths although he stressed the similarity between these overgrowths and those caused by crown gall. "The internal differentiation of masses of non-pathogenic excess callus resembles somewhat the previously described histology of crown gall" (171). There is no evidence as to whether or not this is a similar situation to that occurring in roses.

In roses the initial callus formation in variously infected and mutilated wounds fell into several types. Apart from the simple growth of callus from the ends of rays (Fig. 23) and the development, behind the necrotic plate, from the immature secondary xylem cells, by tangential division, of radial tightly packed columns of thin-walled rectangular cells (Fig. 24) as described by Buck (39) two other types of growth occurred. These were both composed of cells more than twice the diameter of those described above. Single upright strands, sometimes branched, of 3 to 6 large, more or less isodiametric, thin-walled cells, loosely packed or barely touching, appeared over the whole surface of the necrotic plate (Fig. 24). In areas where space for development was restricted large cells of a similar size and appearance appeared as a parenchymatous tissue without any evidence of a regularly orientated plane of division (Fig. 25). These two

types of large celled callus could be similar in origin to the excess callus described by Sass (171) in the quote above. Certainly the cells resembled the histology of transformed crown gall cells.

As it did not prove possible to regularly induce the formation of this large celled callus and as it was usually found in association with the two presumably normal types of callus described by Buck (39) the subsequent development of this tissue was not able to be followed. Cells of similar size and random arrangement with secondary thickening and conspicuous pits were observed in older wounds and graft union tissue.

The appearance of callus formation can vary quite markedly depending on the micro-climatic conditions at the wound surface. In conditions of low humidity phellogen is initiated 3 to 4 cells below the callus surface much earlier in the callus development and callus growth is slower than in high humidity.

With respect to the actual bud graft union this has already been described for roses (39, 40, 180) for Prunus (64) and for Malus (193). Some features in common with bud graft unions are found in descriptions of cleft graft unions (47, 176). A number of workers have described defective graft unions in apples (28, 144, 164, 171, 186) in terms of "callus knots", "vascular nodules" and "callus overgrowths". Most of these workers have considered crown gall as a contributing factor but have generally discounted it on account of the low rate of recovery 1 to 2% from isolation studies of defective apple grafts. The

formation of organ primordia and teratological structures around apple graft wounds in association with crown gall was recorded in 1933 (186). It is worth noting that Riker & Keitt (164) showed that there were two distinct types of malformations present on apple grafts termed by them "crown gall" and "callus overgrowths". These are probably analagous to galls and excessive callus as used in this study. It was from isolation studies of overgrowths, not galls, that only 2% yielded A. tumefaciens.

In the material studied the actual histological development of the bud graft union is not as simple as that described by Buck (39) for similar material. This is due to a difference in the method used for budding in New Zealand to that used by Buck. In New Zealand most commercial operators leave the thin sliver of wood, which is cut with the bud, in place behind the bud shield whereas, with Buck's work, this wood sliver was removed. Development up to the stage of formation of a band (probably more correctly a plate) of cambiform tissue across the face of the stock is similar except no callus forms on the inner surface of the scion xylem behind the bud and scion callus is only formed where the immature cambial derivatives of the scion are exposed. The bridging cambium is formed in a similar manner to that described by Buck except that the scion cambiform tissue differentiates at the exposed edge of the scion cambium rather than across the face of the scion from behind the bud. The resultant union is similar to that

diagramatically illustrated by Mosse and Labern (1944) for apple bud-unions, which are also budded by the "wood-in" method. In roses the bark flap is frequently excluded from the final cambium cylinder, unlike their diagram, due to the bridging cambium arising from stock cambium towards the base of the bark flap.

Both the exclusion of the bark flap and the presence of a gap between the original scion xylem (sliver of wood) and the original stock xylem tend to increase the occurrence of a confused array of vascular nodules and strands in these two areas, amphicribal in the bark flap and amphivasal in the stock xylem-derived tissue behind the original scion xylem. These vascular nodules and strands do not appear to play any essential part nor do they appear necessarily detrimental to a successful graft union. The amphivasal nodules usually do not become fully lignified and cease growth in older unions when the area they occupy becomes filled with tissue.

The inclusion or exclusion of the bark flap in the main cambial cylinder of the union appears to be a factor of pressure applied by the ties around the graft. Variations in patterns of differentiation in the bark flap and in the upper and lower tips of the bud shield apparently due to the application or lack of pressure from the bud ties were in agreement with the findings of Brown & Sax (37).

In the bud unions prior to development of the scion shoots no obviously abnormal condition could be found which could be correlated with subsequent

proliferated growth. The occurrence of exceptionally large randomly arranged cells as already noted appeared only in a proportion of the wounds and bud grafts examined but at no stage did they appear as a major feature of the graft union nor did they appear to add to or detract from the establishment of vascular continuity.

This work was considerably hampered by the inability to transmit proliferation symptoms and hence know whether any particular bud graft union being examined would have subsequently developed proliferation symptoms. The development later in this investigation of a means of determining the probable fate of scion growth (Figs. 10, 11, 12) will serve as a useful guide in future work.

In examination of both early stages of union formation and unions after scion growth or proliferation symptoms had appeared there were a number of unions which were incomplete due to poor placement of the scion bud and inadequate pressure on one side. These usually only established a cambium bridge on one side or part of one side of the bud shield. Older unions of this type usually showed that a second cambium bridge had formed across the inner side of the bud shield thus giving some semblance of a continuous stem cambium in the manner of Brown & Sax's experiments (37) but linking on one side of the loop with the scion cambium. These mechanically poor unions were found both on proliferated and normal shoots and could result ultimately in apparently normal plants.

Examination of older graft unions of both normal and proliferated shoots showed no apparent difference in the establishment of cambial continuity in the region of the cambial bridge and the occurrence of excessive callus on proliferated plants occurred on other tissues independent of cambial continuity. The formation of abnormal tracheary elements in the xylem at the base of the shoot as described with the proliferation symptoms did not appear to affect the graft union itself. This would probably not apply in the case of graft failure due to galling behind the bud shield (Figs. 19 & 20) but early stages in the development of this condition were not examined.

The conclusion drawn from these observations was that neither abnormalities in the actual bud-graft union nor mechanically faulty technique was responsible for proliferation and that the presence of excessive callus was probably a symptom of the proliferation disease rather than a cause of irregularities in scion bud growth. This excludes those occasional cases where disorganised tumor-type growth occurs behind the bud shield (Figs. 19 & 20) and forces it away from the stock so that neither a union is formed nor any shoot growth occurs.

Occurrence of Abnormal Growths:

Teratomatous growths such as are shown by the proliferation symptom can be caused by a wide range of agents. The survey by Bloch (23) is a useful review of abnormal growth. A distinction can be drawn between teratomatous shoots that occur in association

with galls and tissue overgrowths and those that occur independently of massive tissue hypertrophy.

In the latter category are the "witches brooms" in the main caused by fungi in the Exoasceae and the Uredineae and by mites.

Those that are associated with galls and tissue overgrowths also tend to fall into two categories. Galls that are organised in structure with fully differentiated tissues and of more or less determinate growth are known as prosoplasmatic galls. These are generally caused by insects and also some fungi and are often associated with multiple shoot formation. The second type is associated with amorphous galls and tumors of the cataplasmatic type.

Although the proliferated shoots in affected roses do not arise directly from such galls the association of the proliferation symptom with apparently tumorous galls and excessive callus of disorganised structure suggests that this proliferation is due to the occurrence of cataplasmatic tumors.

Again a large number of factors are known to induce galls and tumors many of which on occasion are known to produce multiple malformed stunted shoots (23, 24, 56, 110, 130, 131, 175, 177 et al) with occasionally the development of one or more normal shoots amongst the teratomatous growths in a manner very similar to that occurring in proliferated rose buds. These factors include a wide range of chemical agents, ionizing radiation, viruses, bacteria, fungi, mites, genetic factors and even by removal of the growth

buds (177).

Of these ionizing radiation and chemical agents other than those naturally produced have been ignored as being unlikely to be involved but some consideration has been given to the claims of the other factors as possible causal agents.

Chemical Factors:

Loss of apical dominance is apparent on proliferated shoots although the symptoms go far beyond simple loss of apical dominance and it is worth noting that ethylene, illuminating gas and triiodobenzoic acid have been recorded (8, 201) as forcing development of latent rose buds and basal shoots in glasshouse roses.

The control of bud growth by vascular tissue differentiation (168) indicates that any factor which affects the differentiation of vascular tissue may affect the growth of a lateral bud. The release of lateral buds from apical dominance by cytokinins (169, 170, 172) is a well documented aspect of growth hormone effects. The role of cytokinins in stimulating tissue growth and cell division particularly *in vitro* is too well documented to need detailing here.

The effect of auxins in stimulating callus development and a considerable tumor like growth, consisting of a mass of meristematic, parenchymatous and tracheid elements, when applied to a wounded stem *in vivo*, is also well documented.

The effects of gibberellins are less obviously related to the symptom expression but the effect of gibberellin causing narrow attenuated leaves in rose

meristem culture by Elliott (60) and in this investigation (Q. Fig. 26) suggests it may have an effect on leaf form. The observation that this effect of gibberellin was alleviated by p-chlorophenoxyisobutyric acid (Q. Fig. 27) is of considerable interest in relation to the interactions of these two classes of growth factors although it contributes little to the present investigation.

It is not normal practice to use any form of growth hormone in the bud grafting of roses and any possibility of an exogenous application of hormone is unlikely. It is an often recorded characteristic of transformation tumors, that is, tumors caused by wound tumor virus, the genetically induced tumors of Nicotiana and crown gall tumors that they exhibit autonymous activity of the cells and have a high endogenous level of auxin and cytokinin. That these hormones can express themselves on otherwise normal parts of galled plants by epinasty of petioles, suppression of leaf growth and development of lateral shoots will be discussed later under "Crown Gall". In genetically tumor-prone tissues of Nicotiana hybrids significantly higher levels of auxin are reported (14) than in the tissues of hybrids and species which are not tumor prone.

Apart from the association with tumors increased hormone levels have been reported in a very wide range of pathogenic conditions caused by other bacterial, viral and fungal pathogens so this relationship in itself is not neccessarily significant.

Non-transforming bacteria:

There are many reports of the production of growth factors by soil and epiphytic mocro-organisms rather than by infected plants themselves (63, 103-196, 108, 116, 124, 132, 133, 134, 173). Although a number of these reports cite observable or measurable changes in plant material or tissue cultures due to the effect of the growth factors produced by the micro-organisms while they are epiphytic or in contact with the plant material the yields of growth factors measured would seem hardly likely to cause as extensive a growth modification as is observed in rose bud proliferation.

On the other hand it should not entirely be discounted that the epiphytic population has some influence on wound healing in R. multiflora as this has been indicated by the observation that sterile stem pieces and bud shields without added growth factors or nutrients make little callus growth compared to similar material which has had the same treatment but has proved to be not completely sterile, (M, N, Q).

Although these comments have interesting connotations with respect to the microbial environment of plant propagation it is not proposed that exogenous growth factors whether produced in situ by epiphytic micro-organisms, or by any other means, are involved in causing bud proliferation of roses although levels of endogenously produced growth factors are almost certainly to be involved in symptom expression as is probably the case in many if not most other plant diseases.

Mites:

The possibility of the involvement of an eriophyid mite was not overlooked as some of these mites cause galls and undifferentiated tissue growth. A paper (4) recording the transmission of a rose virus termed rose rosette virus by an eriophyid mite also raised a further possibility although the viral symptoms are quite distinct from proliferation.

Rainbow (159) records in his report on proliferation that a small number of stocks with callus growths were examined for mites, with negative results.

The writer in this investigation did observe 4 or 5 eriophyid mites in total on several hundred specimens handled but certainly not enough to associate them with either the galling or the proliferation symptom.

Fungi:

Isolations for fungal pathogens in the bud unions of a total of 143 plants showing typical proliferation symptoms from 10 different properties were carried out and reported on by Rainbow (160). Of the fungi isolated the most consistently occurring was Leptosphaeria coniothyrium which in 7 out of the 10 properties was found in 80% or more of the unions,

One communication (145) from the United States also suggests this fungus as possibly a contributing factor but it is not clear whether this in fact relates to typical proliferation or merely poor unions.

A number of innoculation trials (152) were then carried out by Rainbow and he came to the conclusion that although L. coniothyrium could be detrimental

to the bud union it was not responsible for causing the proliferation symptom (153).

No other results or evidence as to the involvement of a fungal pathogen is known.

Viruses:

In some respects the implication of a virus as an etiological agent for proliferation disease would seem most attractive. All the symptoms are of types which at least seperately are associated with various viruses of other plants. Viruses which show symptoms of the type found in the rose proliferation condition are generally "yellows type" viruses many of which are now being found to be associated with mycoplasma organisms rather than true viruses. Viruses of the yellows type and diseases associated with mycoplasma are in general leaf hopper transmitted. A vector of this type could account for some apparent anomalies of occurrence and distribution of the proliferation disease.

Of the viruses which show various excessive tissue growth all show hyperplasias of a specific tissue or organ and only one the wound-tumor virus forms more or less true transformation tumors generally on the roots and less frequently on the stems and veins where they may occasionally produce leafy outgrowths (20) although these generally do not produce multiple shooting. Virus tumors are more differentiated and histologically have smaller less parenchymatous cells than crown gall tumors (19).

Like the yellows type viruses wound tumor has a leaf hopper vector and although Black (20) states

that there appears to be little connection "symptomatologically" Nyland and Goheen (1954) list the wound tumor with the yellows type viruses. Unlike all the others in this list wound tumor virus has a known virus particle.

Although wound tumor virus can infect a wide range of hosts there is no report of it occurring on roses nor does there appear to be any report in the literature of yellows type viruses occurring on roses. The well known Mosaic-type rose viruses are obviously not implicated in proliferation although it it worth noting that at least some of them have been relatively easily heat inactivated (154).

Rose viruses are relatively difficult to characterise as roses are noted for their high concentration of polyphenols and their oxidation products, quinones and tannins. On wounding or crushing rose tissue these compounds denature the virus protein coat. When a virus is isolated it is equally difficult to re-infect (46). On this account, uninfected rose cultivars or rose species known to produce symptoms of the virus in question are generally used as indicator plants and graft transmission is used in most routine identification work. There has been a report (66) that strawberry, Fragaria vesca, indicator clones can be used as virus indicators for roses by approach grafts to stolons or the insertion of an excised rose leaf in place of a central strawberry leaflet. HRC - Levin did indicate (153) that they were going to try this technique with proliferation but no further information

has come to hand.

An interesting technique for transmission from rose has recently been published (11) using a patch bark-graft from the rose on to young peach seedlings and after 1 month transmitting the virus from young peach leaves to cucumber seedling cotyledons. This avoids many of the problems with polyphenols and their oxidation products and enables the innoculation of herbaceous hosts. This technique resulted in the consistent isolation of 2 pathogens via peach to cucumber. Both produced virus like sypmtoms on cucumber and were thought to be viruses but one was subsequently found to be a species of Pseudomonas. Unfortunately the symptoms on roses are not adequately described in this paper.

Similar results showing virus-like symptoms produced by bacteria using pear blossoms and cherry buds on cucumber cotyledons have been reported (162) and the bacterium identified as Pseudomonas syringae the causal organism of blast.

It is not shown whether the bacteria were systemic or epiphytic and although in these two cases an epiphytic origin must be presumed until shown otherwise, the systemic existence of P. syringae in cherry trees has been shown by Cameron (40a.).

Despite the recording of P. syringae in roses (35a), its subsequent epidemic occurrence in roses at about the time proliferation started to become a problem (74) and its isolation from some proliferated shoots that had died back (159) the symptoms of blast

are well defined and quite distinct from those shown by proliferation.

It is possible that this bacterium could produce hitherto unrecorded symptoms when systemic infection occurs in a bud graft union of roses. There is, however, no evidence for such a suggestion and the correlation of galling excess callus and proliferation as shown subsequently in this investigation is strong evidence against it.

Rose rosette virus has already been covered under the heading of "mites" (4).

There are two rose viruses which may on occasions be nematode transmitted (41) which are receiving considerable investigation in England (46). The nematode Xiphinema diversicaudatum is capable of transmitting arabis mosaic virus (187) and strawberry latent ringspot virus (86) both of which occur in roses in Great Britain.

As nematode transmitted viruses are also, in general, seed borne with a small percentage of infection it cannot be said with certainty that seedling raised stocks or scions are necessarily free of these viruses. Arabis mosaic virus symptoms are quite distinctive and different to the symptoms under consideration here. Strawberry latent ringspot virus described as small stunted shoot from the scion bud with distortion deformation and yellow flecking of the leaves is similar to some of the symptoms described by Fry & Hammett (68) although they record a chlorotic condition rather than yellow flecking. It does not

resemble the proliferation symptom.

The proliferation symptom is known in England (67, 85, 99) where it is also known as maiden stunt (67, 99). It is clearly recognised as being distinct from strawberry latent ringspot virus and many workers in England do not associate proliferation with a virus. Transmission experiments in England using buds from roses affected with proliferation and budded on seedling stocks did not transmit the disorder (85).

Dr. Frost of Manchester University, where much of the basic research on rose viruses in England is being carried out, was unable to find virus in bushes with proliferation (67). He also propagated infected shoots under mist and found new growth normal although some of the shoots died before producing new growth (67).

Growers in England have been aware of proliferation for the last three or four years and it appears to be on the increase particularly in the Nottingham area and also Kent. Losses up to 80% of one half acre block have been reported (99).

At Shardlow Hall trials have been carried out, using buds from plants showing proliferation, on to two different rose stocks and comparing them with plants budded with unaffected budwood. No difference could be found between plants grown from clean and affected material (99). This is in agreement with work carried out in New Zealand (159, 161) using not only buds from proliferation affected plants but putting them onto stock from proliferation affected

plants. These were compared with buds from apparently healthy plants budded on stock known to produce apparently healthy plants. There was no apparent difference between the affected on affected combination and the healthy on healthy (161). Also of 216 plants produced at PDD Auckland from an affected on affected combination only one produced proliferation symptoms (159).

A graft transmissible disease of roses is described under the name of rose spring dwarf (191). The symptoms reported for this are, in the spring, delayed or dwarf shoots, downward curled leaves and leaflets (epinasty), with yellow vein banding, easily detached leaves and in the autumn mild leaf epinasty and down curling. The symptoms of this disorder also had much in common with strawberry latent ringspot virus (41, 46, 67, 86), rose wilt virus (80, 81, 122) and with some of the symptoms described by Fry & Hammett (68) but not the proliferation symptom.

The symptoms which they (68) attribute to rose wilt virus go far beyond those that were originally described by Grieve (80). The suggestion made by one of them (153) that they may be dealing with a virus complex is no doubt correct and the inclusion of the proliferation symptom probably extends the range of the complex beyond that of viruses.

The description by Grieve (80) of rose wilt virus symptoms is very close to those of verticillium wilt on roses. The opinion expressed by Dimock (54) that it would be difficult to distinguish between

verticillium wilt and the symptoms described by Grieve is quite a valid one nor does the paper by Hammett (84) entirely clear this issue due to the inclusion of symptoms of rose wilt virus in a much wider sense. This probably will not be able to be cleared until the symptoms of rose wilt virus on their own can be critically compared with those of verticillium wilt.

Grieve's original description of the rose wilt virus symptoms (80) does not appear to differentiate between leaves and leaflets but he apparently uses the term leaves to refer to leaflets particularly when he refers to "Leaves recurved on petioles" and "The leaves sometimes have an appearance of being crowded together on the petiole". The latter quote does not in any way seem to imply a shortening of the internodes as is found in the rosetting symptom. Similarly the ease with which the "leaves" drop off in the early stages of defoliation would appear to refer in the first instance to the leaflets. This interpretation would appear to be confirmed by an inspection of the illustrations (80) which shows the recurving confined to the leaflets and a number of places where the leaflets have dropped but the petiole or rather the complete rhachis is still intact on the plant. No epinasty of the petiole can be observed therefore the recurving symptom as recorded by Grieve is confined to the leaflets. This is clarified in his subsequent paper (81) particularly with respect to the recurving of the leaflets and should not be confused with a natural curvature of the rhachis which occurs normally

in very young emerging rose leaves.

Briefly the essential symptoms described by Grieve (80) in sequence of occurrence are:-

1. On young or recently mature shoots the leaflets become recurved brittle in texture and tend to fall off.

2. Defoliation commences at the tip of the young stems and progresses downwards.

3. After an interval of about one day the tips of the young stems discolour and die back. The rest of the young stem becomes translucent yellowish green for a day, then the colour intensifies at the base of the stem and turns a brownish black.

4. The whole of the stem gradually discolours and dies back. The dieback may extend into the older wood from which the shoots arise.

5. The plant may send up one or more strong shoots (Watershoots) which soon show signs of the disease and repeat the symptoms above.

6. The plant may apparently recover for one or two seasons before showing the symptoms afresh.

The time course of the development of these symptoms should be noted with respect to the speed with which they progress.

This appears to be in agreement with the development of symptoms in the experimentally innoculated plants in the laboratory and not with those growing in the open. The symptoms in the plants innoculated in the open are much less severe and appear to be confined to either the characteristic recurving of the leaflets

or dying back initiating in the young shoots (80, 81). This would be in agreement with the symptoms described by Brundrett (38) and by Elliot (59).

The symptoms described by Fry & Hammett (68) on mature plants go considerably beyond those of Grieve and include a further range of symptoms on mature wood. They have returned to the description that "leaves" are epinastic and from advice to nurserymen and lectures by Hammett it is clear that they also include simple epinasty of the petiole in young plants among their range of symptoms. Their "epinastic balling" illustrated in Fig. 4 of their paper closely conforms with the recurving of the leaflets and crowding on the petiole as described by Grieve but this is confused in the text (Ref. 68 : p. 738, 1st para.) with a rosetting symptom which occurs as a result of gross reduction or virtual absence of internodes on growth from lateral buds on the previous season's stems. The loss of apical dominance would appear to refer to the fact that the rosette symptom occurs from many lateral buds on the previous season's growth as compared with the normal condition when 1 to 3 lateral buds only grow to form normal shoots.

It is not clear whether the sentence "Such leaves are brittle and soon fall" refers to the recurved leaflets on young shoots with normal length internodes or the leaves of rosetted shoots with shortened internodes occurring laterally on older growth or both.

The references to dieback are also not clearly referred to either or both of these distinct types of

symptoms.

From the writer's knowledge of these symptoms in New Zealand the leaves of rosetted shoots are not particularly short lived nor do they have recurved leaflets in the same sense as occur on the long younger shoots and as described by Grieve. Similarly dieback in New Zealand is generally associated with the mature or previous season's growth which shows the rosetting symptom and shortened shoots from lateral buds.

It would thus appear as if there were two separate syndromes involved in the symptoms on mature plants.

1. The characteristic recurving and brittleness of the leaflets on young growths of mature plants which either may become more or less normal as the shoot ages on outside grown plants or less commonly in field conditions (rarely in the writer's experience) may die back. In other words the rose wilt virus symptoms of Grieve (80).

2. The rosetting symptom occurring on growth from lateral buds of mature or previous season's shoots. The leaflets of the rosetted shoots are not recurved but are flat more or less reduced in size and are not noticeably brittle or easily shed while young. Die back of these older shoots and often consequently of the rosetted laterals frequently occurs slowly and to varying degrees.

Both these syndromes may be observed on the one plant but not invariably so. As both are of high incidence in New Zealand it is not surprising that

they should at times both be seen on the one plant and if both are caused by agents which are graft transmissible both could occur as the result of transmission experiments.

If the rosetting symptom and die back of mature wood on which it occurs is distinct from rose wilt virus in the sense of Grieve it may be a symptom of mature plants which have been affected with proliferation in their young stages. No effort has been made to investigate this possibility.

These observations would appear to support the suggestion (153) that the symptoms in mature plants described by Fry & Hammett as rosc wilt virus are in fact caused by a complex and not by rose wilt virus alone.

From the writer's knowledge of young nursery plants growing away in the spring from the scion bud a number of symptoms can be observed some of which appear to fall into separate syndromes attributable to seperate etiological causes.

The typical recurving of the leaflets associated with brittleness and ease of shedding as in the rose wilt virus syndromecan be easily recognised and no doubt is associated with this condition in the mature plant. These shoots do not normally die back.

The proliferation symptom already fully described in this paper (pages 23-32 : Figs. 8 & 9) and well illustrated by Fry & Hammett (Figs. 1 & 2) is easily recognised in its typical form and has never been shown to be graft transmissible despite a number of

attempts (85, 99, 159, 161). No evidence exists to associate this symptom with any symptoms known to occur in mature plants although possibly if the rosetting were shown to be of similar etiological origin this would explain the appearance of rosetting in plants thought previously to be healthy and the occurrence of proliferation becoming a problem in nurseries over the same period as "die back" of the type associated with rosetting became a problem in recently transplanted roses.

The symptoms of pinch off as described by Roberts (165) also occur without multiple shooting but these may prove to be part of the proliferation disease syndrome as a similar pinching off of proliferated shoots frequently occurs.

Stunting of the young scion may also occasionally occur as noted by Rainbow and Powell (160) and this condition is distinct as it has more or less normal leaflets with thick veins being neither recurved nor vestigial.

Various colour abnormalities appear on young growing scions but the reasons for occurrence of these is uncertain.

The occurrence of a very slight degree of true epinasty of the petiole without recurving of the leaflets is often noticed on most of the very young and very actively growing otherwise apparently normal scion shoots. This quickly disappears as the growth becomes firm and is probably only an expression of the very rapid elongation of the shoot.

The symptoms on nursery plants attributed by Fry & Hammett (68) to rose wilt virus include most of those described above although it should be pointed out that in their transmission work using an indicator scion that only "epinasty", presumably recurving of the leaflets, is recorded as having been transmitted.

At a field day organised for interested parties to view these transmission experiments at PDD Dr. Rainbow and the writer who had both been working on proliferation in Levin were present. We both agreed that the symptoms which had been transmitted were quite distinct from those which we knew as proliferation.

Although Fry & Hammett use the term proliferation to refer to what they consider one of the symptoms of rose wilt virus the tendency has recently crept in, in general usage, to use the terms proliferation (disease) and rose wilt virus as synonymous. This is unfortunate and is tending to cause confusion.

As the main theme of this investigation is proliferation and there appears to be no substantial evidence that it is caused by a virus we will look at the evidence with respect to its being caused by an organism which also causes galling.

Proliferation, galling and excessive callus:

In view of a notable failure by a number of workers to transmit the proliferation symptom by bud-grafting (67, 99, 159, 161, 165a) and in view of the failure to isolate from the bud unions a fungal pathogen (160, 161) or a bacterial pathogen (153, 159) likely to

cause the disease, the apparent correlation of galling and excessive callus with the occurrence of proliferation and bud failure (A, B, C, D, E, G, I.) remains the major indication as to a possible cause.

There is no doubt that many debilitated commercial clones of R. multiflora understock which show the wide range of symptoms attributed to rose wilt virus by Fry & Hammett (68), show reduced vigour as shown by their work and by the experiment described in appendix E (see Fig. 1). However this "infected" stock does not have a reduced bud take or yield of bushes, except as may be caused by extreme unthriftyness of the stock plant at budding or by the presence of galling (E). There is some indication that the lack of vigour may be in part overcome by subsequent vigorous growth of a healthy scion (E) (Fig. 1).

The finding by Fry & Hammett (68) that galling and excessive callus is not associated with rose wilt virus has been further substantiated in that, although galling and excessive callus occurs on known rose wilt virus infected clones and all observed "probably infected" commercial clones, it also occurs with a similar degree of incidence on seedling clones (K) and by observation on Iowa 60/5, (ISU 60/5), a specially selected and carefully maintained virus free stock from America. This latter observation is of particular interest in view of the finding that ISU 60/5 is particularly resistant to crown gall (25) although the resistance varies depending on the strain of Agrobacterium used to innoculate.

It would appear therefore that all observed clones of R. multiflora irrespective of whether they are infected with rose wilt virus or not are susceptible in some degree to galling. The incidence of galling may vary greatly, even with the same stock clone, from season to season, area to area and even from month to month with respect to the time of planting of the stock cuttings (E, H.).

The correlation of above ground galling and excessive callus symptoms with the incidence of proliferation and bud failure seems indisputable and the thought that this may be merely due to the absence or reduction in vigour of a growing top is contrary to observed evidence (I, J.). The tendency to galling and excessive callus is independent of budding although there is some evidence that the expression of the symptom may be suppressed by the presence of a successfully grafted vigorous scion top (I, J.) and conversely failure of a graft union does not necessarily result in excessive callus growth (E, I.). There is little doubt that leaving a stock top intact suppresses ground level and above ground galling.

As the evidence indicates those plants which have been galled at the base of the original stock cutting tend to be predisposed to forming excessive callus at wounds above ground (G). That the galling at the base of the original stock cutting is initiated either at the time of inserting the cutting or shortly afterwards is apparent by the extensive galling present on the young establishing stocks (H, K) (Figs. 11 & 13).

This is supported by the lack of roots on those sides of the base of the stock where the remains of galls are apparent on older plants (G, J) (Figs. 16 & 17) which corresponds with the position of root initiation in relation to galling in younger stocks (H, K). Thus galling precedes or coincides with root initiation.

The expression of slight but distinguishable symptoms, on the young tops of galled stocks (H, K), which are different in appearance, to the tops of young ungalled stocks (Fig. 10) and those which are merely establishing poorly for various other reasons, indicates that the galling is causing some change in the stock other than mere mechanical interference due to poor root establishment.

Although some practice is required in recognising the symptoms in the young stock tops as they are relatively slight and vary in degree, this may prove a useful tool in determining which stocks are predisposed to bud failure and proliferation (K). The failure to transmit proliferation and hence know in the early stages of bud graft union which plants are going to show proliferation symptoms, has been a major obstacle in this investigation, particularly with respect to microscopic examination for early symptoms of the disease. It would appear however that the first symptom of infection or of a plant being predisposed to developing proliferation is the galling at the base of the original cutting.

The progression of the appearance of symptoms appears to be consequent on the initial galling in the

following sequence.

1. Root initiation at the base of the cutting tends to be on the side of the cutting away from the galling and inversely proportional to the extent of the galling (H, K) (Fig. 13).

2. The first growth of the stock top shows slight symptoms ranging from distinct to obscure and the degree of expression of the top symptoms appears more or less proportional to the extent of the galling (H, K) (Figs. 10, 11 & 12).

3. Subsequent development of the stock top may appear normal.

4. The spread of the root system around the base of the cutting remains restricted to the position of root initiation at least for the first 2 years (G, J) (Figs. 16 & 17).

5. If the stock top is left intact no further symptoms occur (I).

6. Removal of the stock top in the spring causes expression of the excessive callus symptom on infected stock only even if not budded (I, J). This may be accompanied by galling but it is uncertain whether the expression of galling above ground may not require some additional stimulus such as re-infection.

7. If the plants have been budded prior to spring removal of the stock top the growth of the scion on infected plants may occur in a number of ways.

(a) Galling and sometimes excessive callus may occur behind the bud shield forcing it away from the stock with ultimate death of the whole bud shield (Figs. 19

& 20). (b) The bud may just start to move and then fail although the bud shield remains alive for some time, (bud failure). (c) The bud may grow to give typical proliferation symptoms to varying degrees with ultimate death of the proliferated shoots even though a normal shoot or shoots with a normal vascular connection may become established and survive usually arising to the side of the proliferated shoots. (d) With vigorous scions some may develop apparently normal growth and grow into normal plants apart from root spread (J).

With regard to excessive callus in conjunction with the symptom expressions above, this tends to be inversely proportional to the extent of normal scion growth. The immediate development of a normal scion top may suppress the formation of excess callus on infected stocks. The development of normal shoots on scions which initially show proliferation tends to stop further callus formation beyond that formed in the proliferation stage. Bud failure and proliferation are usually accompanied by full expression of the excessive callus symptom with or without galling.

The first condition, 7(a) above, differs somewhat from the others in that a normal graft union between the bud shield and the scion does not appear to develop and galling occurs in this region (Fig. 19). This is possibly caused by some additional factor such as infection occurring between the bud shield and the scion. This is supported by the frequent observation of intermediate conditions where part of the bud shield

is lifted by gall formation and part forms a normal union (Fig. 20).

It should be noted that this analysis of symptom occurrence relates to plants from which the stock top has been excised in late winter and early spring. If the stock top is removed during summer and early autumn there is little symptom expression either of excessive callus or of proliferation or bud failure. This is no doubt due to different growing conditions and the plant being in a different physiological state of growth at this time of the year.

This method has been used commercially as a means of avoiding proliferation and bud failure but the resultant plants are generally inferior to those produced by a spring heading of the stock.

Having thus analysed the occurrence and correlation of symptoms the evidence is strongly in favour of the galling at the base of the original stock cutting being the first observable symptom of proliferation disease.

In order to define the epidemiology of the etiological agent causing the appearance of these symptoms an examination of the occurrence of basal galling (G) and above ground symptoms (B, C, F, G, J) in relation to adjacent plants in the rows reveals that the incidence is at random. Thus there is little or no infective spread from one plant to another and the incidence of infection appears to be determined at the point in time at which the cuttings have been planted although the expression of symptoms as outlined

above may be dependent on subsequent factors. This random occurrence also precludes the suggestion which some workers have made from observation without analysis (153, 159) that the occurrence of prolferation appears to be in groups along the row which might correspond with the sequential use of buds on infected budsticks.

In the course of this analysis it was found that the stunt symptom, as recorded by Rainbow (160), although of relatively low incidence, showed strong evidence of grouping in a manner which would be commensurate with the use of infected budsticks (F). This supports the validity of the method used as a means of determining correlation with the use of infected scion material.

The occurrence of such a random pattern of distribution could be determined either by the random planting of systemically infected stock cuttings or by a hit or miss infection of the cut end of the stock with a widely dispersed, weakly invasive or occasionally pathogenic organism either in the soil or carried on the cuttings.

With respect to the possibility of systemically infected stock cuttings there is evidence against this as already described in that galling and proliferation are found in similar patterns and frequency of occurrence in stock cuttings obtained from various sources including seedlings and also that cuttings from affected plants budded with buds from affected scions did not show any transmission of proliferation.

As the evidence and the elimination of alternatives seems to support the involvement of a widely dispersed, weakly invasive or occasionally pathogenic organism and as the type of galling at the base of the cutting is similar in appearance to the galling caused by Agrobacterium tumefaciens an examination is made as to the possible involvement of this organism.

Crown Gall:

Attempts during the course of this investigation to isolate A. tumefaciens from bud-graft unions, excessive callus, above ground galls and galls at the base of stock cuttings have all been unsuccessful. Similar results have been obtained at PDD from the examination of bud-graft union, from the excessive callus there and at the point of excision of the stock top (159, 153, 55). However with respect to the galling at the base of R. multiflora cuttings several workers have isolated bacteria from this galling which appear to be crown gall bacteria (55, 141) although precisely what identification tests were carried out is not known. Some at least of the isolations failed to produce gall on test plants, such as tomato, normally used for the identification of crown gall.

This would suggest that Agrobacterium is in fact present at least in the basal galls and that either the writer failed to isolate due to inadequate technique or in fact did isolate the bacterium but the re-innoculation conditions used were unsuitable for developing galls and hence showing the identity of the organism. In the case of test plants other than

roses the strain of Agrobacterium may be one that does not cause symptoms on the species tested. The report by Maas-Geesteranus and Barendsen (137) of a condition causing stunting of glasshouse roses caused by A. gypsophilae (Brown) Starr & Weiss, which produced galling symptoms at the graft union intermediate between the tumors caused by A. tumefaciens and the hairy root symptoms caused by A. rhizogenes may be pertinent. Innoculation of stem bases of tobacco, Pelargonium zonale and tomato with A. rhizogenes did not produce disease symptoms (137). Their methods were used in a number of isolations in this investigation but without success in reproducing symptoms in roses.

Before looking in detail at recent research on Agrobacterium the tumor inducing principle and transformation experiments relating to this field an examination of the well documented characteristics of crown gall shows many features in common with those shown by proliferation.

Soil-borne pathogenic micro-organisms tend to fall into three groups (10):- those in which the bulk of the population tend to occur in the host plant and the soil-borne population is a rapidly declining one: those in which the bulk of the population occur largely in the soil as a saprophytic population and occasionally cause pathogenic effects on plants and an intermediate group which are dependent on suitable hosts for most of their population build up but which exist saprophytically in the soil with only a slow decrease in population or in favourable conditions may increase

in the soil phase. Agrobacterium would be included in this latter intermediate category (10). Where it exists in an area carrying susceptible hosts, which may or may not be the cultivated crop, it tends to be widely dispersed with the total population in a state of flux, depending on increase from the breakdown of infected host tissue or saprophytic increase under suitable soil conditions and has a relatively slow decline in population when these factors are not present. The soil population of Agrobacterium includes non-virulent strains as well as strains which may vary in degree of virulence on different hosts.

It can be seen that such a widely dispersed population varying with soil conditions and consisting of various virulent and non-virulent races would be capable of infecting a crop in a random pattern varying from area to area, year to year and month to month with a seasonal flux depending on soil conditions (52, 53). This would be particularly so when, as is the case with Agrobacterium, infection is dependent on wounding below soil level and such major wounding on rose stock occurs at one point in time, that at which the cutting is pushed into the ground.

Although such random distribution of primary infection is uncommon in permanent plantings with little total cultivation, it occurs frequently with some soil-borne pathogens in areas which are regularly cultivated and cropped (10).

A well documented feature of A. tumefaciens is its ability to induce secondary tumors and tissue growth

away from the site of infection without the involvement of the bacteria and even at the primary site of infection the presence of the bacteria are no longer essential for continued autonymous tumorous growth once the initial transformation has taken place.

In the case of the rose stock, at least the excessive callus and possibly the above ground galling, which occur consequent on the occurrence of galling at the base of the stock and consequent on removal of the stock top, may well occur in a similar manner to the formation of secondary tumors. This would account for at least the failure to isolate the pathogen from the bud-graft union and from the callus overgrowths.

An increase in tumor growth is described in many papers subsequent to removal of lateral buds or decapitation of an infected plant and this is precisely what is done in rose propagation by the usual budding method.

Although secondary effects of crown gall transformation tend to be produced proximal to the point of primary infection they are frequently also produced distally particularly when gross mutilation such as disbudding or decapitation of the plant is carried out.

Induction of teratoma (32, 33, 34, 56, 130) may be induced in tomato, tobacco and other plants which have a high genetic capacity to initiate adventitous buds. In tobacco they have been found to arise regularly following innoculation of the host with certain strains of crown gall bacteria of moderate virulence but tumors only are formed with highly

virulent strains (33a). The formation of teratomas appears to be dependent on the interaction of a number of factors including the strain and virulence of the bacteria, the relative position in the plant of the tumor, the degree and manner of mutilation of the host and the inherent genetic capacity of the host species to form adventitious buds.

Although neither R. multiflora nor the common garden cultivars which show proliferation have been observed to form adventitious shoots, the involvement of the bud meristem and the shoot primordia in the axils of cataphylls in the formation of the teratomatous proliferated growths, would appear to be a source of shoot primordia other than of adventitious origin.

That at least some morphological changes can occur as a result of infection by crown gall has been reported (138) on grapevines where infection led to more or less pronounced deformations of the rootstock, stem, pruned shoots and fruiting canes.

The observed relationship of high sap flow at time of budding to increased incidence of proliferation (74, 143, 159) could appear to relate to the involvement of wound sap in crown gall transformation but the evidence indicates that primary infection occurs at the base of the cutting not the budding wound. However high sap flow could delay graft union formation and increase expression of the symptoms in some scions that might otherwise have grown strongly and suppressed above ground symptoms (I, J).

Although the parallels drawn above, between

proliferation and crown gall, are purely circumstantial and will be, until the causal agent of proliferation can be identified, isolated and used to induce the condition, the apparent similarities in symptom development serve as a useful model of what sequence of events may be taking place between galling of the cutting and the various forms of symptom expression which may develop in the proliferation disease.

It would seem unlikely, that with the known association of crown gall with roses for many years without any recorded occurrence of proliferation (140, 145), that the organism responsible for proliferation will be exactly the same as that known to cause crown gall on roses in earlier reports. Whether it will prove to be a different strain of A. tumefaciens or at least a strain of different virulence or some closely related species carrying a different transformation factor remains to be shown.

Recent research in this field is showing a change of approach with respect to both the problem of crown gall induction and to the classification of Agrobacterium and related genera based merely on host symptom expression.

Recent research on Crown Gall:

The presence of lysogenic phages in crown gall bacteria has been known for more than 40 years (146). The suggestion that transmission and transformation of cells to crown gall tumor tissue was due to a virus was first made by de Ropp in 1947 (49) based on the ability of bacteria free crown gall tumor tissue to

transform adjacent normal cells. In the early 1950s a number of workers conducted experiments to show the presence of a filter passing agent capable of inducing tumor formation. Some failed (17) and the same and other workers met with some degree of success (119, 120). Controversy raged until it was shown that some of the techniques used had allowed small numbers of bacteria to pass the filter. Beardsley in 1955 and subsequently (15, 16) and others (181) published papers on the relationship between phage production by lysogenic strains of A. tumefaciens and virulence although it was found that the phage alone does not induce tumor formation.

Reports that exposure to short wavelength ultraviolet irradiation increases the infectivity of A. tumefaciens (88, 89), despite 50 to 90% reduction in viability of the bacteria, was taken to imply a change in the DNA of the bacterium.

The report in 1968 that bacteriophage activity could be found in homogenates of crown gall tissue which had been grown in vitro for two years (155) implicated the involvement of the phage itself in transformed tissue. An electron microscope study of tumorous tissue reported the previous year (79) had however failed to show any etiological agents or alteration of fine structure peculiar to the tumor cells.

Small doses of X-rays on sterile cultures of crown gall tissue reduce the rate of growth and stronger doses prevent further growth. The grafting of crown gall tissue (with the stronger dose of X-rays) to

healthy tissue induces the adjacent healthy tissue to become tumorous (1). This infers that although the X-rays prevent the growth of the irradiated tumorous tissue they do not prevent the release of a tumor inducing principle possibly analagous to the release of a phage by irradiating lysogenic bacteria.

Kurkdjian, Beardsley and others (127, 128, 167) examined with the electron microscope the presence of various phages in A. tumefaciens bacteria. They found that in the bacteria the phages are situated entirely in the nuclear regions and also that attenuation of virulent bacteria with glycine inhibited the bacteria but had no apparent effect on phage replication thereby implying the necessary role of the bacteria as well as the phage for crown gall transformation.

The appearance of phages of A. tumefaciens at the place of innoculation for induction of crown gall tumors between the 6th and 17th hour afterwards is also reported (127).

The first report of electron microscope observation of A. tumefaciens phages in situ in transformed crown gall cells (97) further confirms the evidence that they are present in transformed tissue. The failure of earlier workers to observe phages in transformed tissue is probably explained by the very low number of phages found in such tissues. Ten grams of transformed tissue only yield between 3 and 70 plaques (190). This however is in conformity with the number found in animal cells transformed by a DNA

virus such as the S.V. 40 or the polyome virus which rarely produces the free virons although it contains the viral genetical material.

The further reporting of phages in transformed crown gall tissue by Tourneur & Morel (190) includes transformed tissue which had been cultivated *in vitro* for almost 25 years. They give a description of the shape and size of the phage which is the same from all tumor tissue cultures observed and from the bacteria.

An attempt to correlate tumor production with phage release (38) showed that lysogenic *A. tumefaciens* were atypical in that UV irradiation produced an immediate release of what was shown to be preformed phages as well as the more normal delayed release following *de novo* phage synthesis.

A phage obtained from raw sewage to which a strain of *A. tumefaciens* acted as a homologous host was used to show that the immersion of tomato roots in a lysate containing the phage resulted in uptake and translocation of the phage into root, stem and leaf tissue within three hours (27). Although the phage shown to be translocated was not the one implicated in crown gall transformation the translocation of a phage and its longevity in the leaves for 168 hours and in the roots and stems for more than 336 hours is of interest as being possibly analogous to a step in secondary tumor formation by crown gall. That phage absorbtion prior to bacterial innoculation significantly reduced gall growth (27) suggests that the phage may have been showing some competitive inhibition or

indirect interference at the point of bacterial attachmen to a specific wound site as indicated by Lippincott & Lippincott (136).

There have been confusing reports with respect to the inducing of a tumorous transformation by treatment with DNA extracted from A. tumefaciens some workers claiming to have done so (125) and others who have failed (18). Some clarification of the situation came from the finding that although a phage of A. tumefaciens cannot alone induce crown gall transformation DNA extracted from the phage can (129). Possibly extracts of DNA from the bacteria containing also phage DNA may give tumor induction whereas extracts containing bacterial DNA alone does not and this would account for the conflicting reports.

The paper by Stroun et al (185) reported that after dipping tomato stems in a suspension of A. tumefaciens the formation of RNA in the plant cells showed a considerably higher percentage of hybridisation with bacterial DNA than RNA extracted from the bacteria themselves. This interesting result which indicates a higher rate of bacterial DNA transcription in the plant than in the bacteria makes no mention of possible involvement of phage DNA.

Similar homologies between A. tumefaciens DNA and RNA from crown gall cells have been reported in other RNA - DNA hybridisation studies (142) and between tumor cell DNA, bacterial DNA and DNA of stressed normal cells (157).

Further papers (182, 183, 184) confirm the release

of bacterial DNA into the plant from bacteria and its subsequent transcription in the host cell accompanied by a partial or total shut-off of host cell DNA transcription. The discussion as to possible explanatory hypotheses (184) does not appear to give much consideration to the possibility that the phage DNA may be only loosely integrated with the A. tumefaciens DNA. Purified bacterial DNA which migrates in the host and replicates but is not transcribed may be an extract which has been separated from the phage DNA. The involvement of the transcription of exogenous DNA in the early stages of tumor induction is strongly supported by the inhibition of tumor formation after early treatment with rifamycin, an inhibitor of DNA - dependent RNA polymerase.

The report (90) of the enhancement of A. tumefaciens infectivity by mitomycin C, an inhibitor of DNA synthesis would seem to infer that DNA replication is not necessary.

The experimental model of crown gall transformation constructed by Guille & Quetier (83) lays particular emphasis on the increase of sequences of DNA high in guanine and cytosine in cells which are wounded or under stress and that these show partial homology with A. tumefaciens DNA and bacterial DNA (157). Evidence that these sequences which are high in G + C quantitatively regulate genetical information (98) and the directly comparable relationship with tumor tissues of animals transformed by DNA virus (69) would lend support to Guille & Quetiers model (83).

However the repeated evidence of the involvement of a phage in crown gall transformation and the presence of the phage in virulent strains but not in non-virulent must to some extent negate overmuch stress on the involvement of the bacterial DNA as such.

This is supported by a number of papers in which the transference of the ability to initiate tumors has been transmitted not only to avirulent strains of A. tumefaciens but to other related bacteria including Rhizobium (111, 112, 113, 114, 115, 119, 120).

Although some species of Rhizobium show a 50% DNA homology with A. tumefaciens and it may be that it is this homologous part of the bacterial genome which is involved together with the phage in tumor transformation.

The identification of plant pathogenic bacteria solely on the symptoms which they cause on susceptible hosts may separate them with respect to the important point under consideration at the time but does not necessarily classify them in homogenous groups (107).

Although possibly not of direct importance to this investigation the uptake of DNA by some bacteria (3) and the transference of bacterial DNA and phage DNA to higher plants as already briefly described raises interesting questions as to the possibility of bacterially mediated or phage mediated transformation being the mechanism of transformation in higher plants of the type being pioneered by Hess, (92, 93, 94, 25).

Conclusion:

It has not been possible to determine the causal agent of proliferation disease or to transmit it experimentally.

The apparent characteristics of the disease syndrome based on the association and occurrence of symptoms has been described.

Of the wide range of possible causal agents that which most closely fit the evidence is the known characteristics of the crown gall syndrome.

Classical crown gall has been known for many years on roses prior to the appearance of the proliferation disease and there does not appear to have been any change in the circumstances of the occurrence of typical crown gall.

It is therefore proposed that the evidence accumulated supports the assumption that rose proliferation disease may be caused by a particular strain of Agrobacterium tumefaciens or some bacterium closely related to it.

APPENDICES.

A. Incidence of galling and excessive callus in relation to bud growth and bud failure.

A block of the cultivar 'Irish Mist' which had been planted as stock cutting at Levin in winter 1967, budded in February 1968 and the stock headed back in August 1968 showed a high incidence of galling and bud failure.

In order to determine the incidence of galling and its relation to bud growth and bud failure seven plots of ten plants each selected at random were recorded.

A total of 51 plants were recorded as galled with a plot mean of 7.29 and a standard deviation of $\pm$ 1.60.

The relationship of the occurrence of galling with those buds which either failed, grew or became proliferated is shown on Table II. It is obvious without analysis that those which showed galling symptoms either failed or proliferated with one exception which only showed slight galling. Similarly those which were not galled grew with three exceptions all of which were very thin unthrifty stocks.

The correlation between galling and failure of the buds is obvious although these observations cannot show whether the buds failed as a result of the galling or whether the galling was resultant on the bud failure.

Although the extent of galling on the individual galled plants was not measured the galls were removed from all the galled plants and the total fresh weight of all the galls determined. Some idea of the extent

of galling can be obtained from the total weight of 283 grams or an average per galled plant of 5.55 grams.

Summary:

(1) Extensive galling occurred on stocks on which the buds had failed to grow but not on those on which the buds had grown.

(2) It was not shown whether the galling occurred as a result of the bud failure or the buds failed as a result of the galling.

Table II:

Correlation of galling with buds that either failed, proliferated or that grew normally.

	Failed	Prolif.	Grew	Total
Galled	48	2	1	51
Not galled	3	0	16	19
Totals	51	2	17	70

B. Analysis of observations on severely proliferated and galled block of roses on a Waikanae Nursery.

These observations were not made on a replicated experiment but were a detailed recording of plants in sequence along two rows chosen at random. These rows had been budded with the cultivars 'Attraktion' and 'Joyfullness'. The observations were made on a severely proliferated and galled block of roses at a commercial nursery situated near Waikanae. The plants were not able to be lifted for inspection of the roots and the details recorded were confined to the presence of galling at ground level, at the budding wound and at the point of excision of the stock top together with details as to whether the grafted bud grew normally, showed proliferation or failed to grow.

An analysis of the cultivar 'Attraktion' was carried out to determine the correlation of galling with proliferation and bud failure. As Table IV shows there was a close correlation between galling and proliferation.

In addition the distribution of affected plants along the row was analysed to determine whether there was any indication of association or affected plants occurring in groups. There could be two possibilities which could cause linking or grouping of affected plants.

If either bud failure or galling were caused by a pathogen, that spread to give secondary infection in healthy plants adjacent to those infected, the

occurrence of groups of infected plants would be found showing a greater degree of association of infected plants than would be expected by random infection.

A second possible cause of a greater than random degree of association of infected plants could be due to transmission through the scion material. Grafting buds are normally taken in sequence from the one piece of scion wood as the budding operator works along the row. Therefore if the causal agent of a symptom is scion transmitted the symptoms should tend to occur in groups corresponding to the use of infected sticks of scion wood. Each piece of scion wood usually carries 3 to 10 scion buds which would show significantly greater degree of association with scion transmission than would be shown with a random cause.

The method used for determining whether there is a greater degree of association than would occur at random was to calculate the expected number of pairs that would occur at random. $p = \frac{x\,(x-1)}{n}$ and its standard error $\pm\sqrt{p.}$ Where p = the expected number of random pairs, x = total number of affected plants, n = total number of plants observed. The actual number of pairs observed are counted and compared with the expected number.

From Table V. it can be seen by inspection that neither the occurrence of proliferation alone, galling alone nor proliferation with galling and bud failure with galling taken together showed any significant departure from that which would be expected with a completely random occurrence.

In the same nursery the second random choice was the cultivar 'Joyfullness' which was recorded and analysed in a similar manner. It can be seen from Table VI. that of those plants which were galled a greater proportion failed and a lesser proportion proliferated than was the case with the cultivar 'Attraktion', Table IV. If bud failure on galled plants can be regarded as an extreme expression of proliferation the correlation of galling with affected buds and the correlation of lack of galling with those buds which grew normally is apparent in Table VI.

A tabulated comparison of the two cultivars with the main categories expressed as a percentage of the total recorded in each cultivar shows clearly the similarity in pattern of occurrence (Table III.) Again as shown in Tables V. & VII. the plants showing symptoms show no significant departure from a random occurrence along the row.

At this nursery it was noted that the stock stems were very thick compared with usual nursery stock being generally 2 to 3 cms. in diameter. The amount of galling and overgrowth tissue at the top of the stock where it occurred was correspondingly massive which contrasted strongly with those stocks which did not show galling.

Summary:

(1) The presence of galls is associated with either bud failure or the occurrence of proliferation.

(2) A comparison of symptoms on two varieties indicated that galled plants tended to either fail or proliferate.

(3) There is no evidence of spread of galling or proliferation from infected plants to adjacent plants.

(4) There is no evidence of association of either galling or bud failure or proliferation with transmission from infected scion sticks.

Table III:

Percentage incidence.

	'Attraktion' %	'Joyfullness' %
Galled and failed or prolif.	57.75	63.89
Galled and grew	9.86	8.33
Total galled	67.66	72.22
Not galled and failed or prolif.	5.63	5.56
Not galled and grew	26.76	22.22
	100	100

Table IV:

Correlation of galling with buds that either failed, proliferated or that grew normally. Cultivar 'Attraktion'.

	Failed	Prolif.	Grew	Total
Galled	4	37	7	48
Not galled	3	1	19	23
Total	7	38	26	71

Table V:

Test for grouping of affected plants. Cultivar 'Attraktion'.

Types of symptoms	Total plants	Number affected	Expected random pairs	Std. Error	Observed pairs
Prolif.	71	38	19.8	± 4.45	22
All galled plants	71	48	31.77	± 5.64	32
Galled and failed or prolif.	71	52	37.35	± 6.11	37

Table VI:

Correlation of galling with buds that either failed, proliferated, or that grew normally. Cultivar 'Joyfullness'.

	Failed	Prolif.	Grew	Total
Galled	12	11	3	26
Not galled	2	0	8	10
Total	14	11	11	36

Table VII:

Test for grouping of affected plants. Cultivar 'Joyfullness'.

	Total Plants	Number affected	Expected	Std. Error	Observed pairs
Prolif.	36	11	3.06	± 1.75	3
All galled plants	36	26	18.06	± 4.25	16
Galled and failed or prolif.	36	23	14.05	± 3.75	14

C. Analysis of observations on a lightly affected block of roses on a Paraparaumu Nursery.

These observations were not made on a replicated experiment but were a detailed recording in the manner of appendix B of the variety 'Western Sun' in a row chosen at random in a commercial nursery situated at Paraparaumu. Compared with the nursery in appendix B this nursery had a considerably higher percentage of buds which had taken successfully and grown into normal plants. It was noted that the stock stems were relatively thin being 6 to 15 mm in diameter and the size of galls and overgrowths was proportionally smaller than in appendix B.

An analysis of the correlation of galling with buds that either failed, proliferated or grew normally (Table VIII) showed that those that were galled showed symptoms of either proliferation or bud failure and those that were not galled in the main grew into normal plants.

The distribution of affected plants along the row was tested by the method in appendix B (Table IX) to determine whether the grouping of affected plants exceeded that expected from a random distribution. As can be seen from the Table there is no significant departure from a completely random distribution of affected plants.

Summary:

(1) The presence of galls is associated with either bud failure or the occurrence of proliferation.

(2) There is no evidence of spread of galling or proliferation from infected plants to adjacent plants.

(3) There is no evidence of association of either galling or bud failure or proliferation with transmission from infected scion sticks.

Table VIII:

Correlation of galling with buds that either failed, proliferated or that grew normally. Cultivar 'Western Sun'.

	Failed	Prolif.	Grew	Total
Galled	18	11	2	31
Not galled	3	1	38	42
Total	21	12	40	73

Table IX:

Test for grouping of affected plants. Cultivar 'Western Sun'.

	Total plants	Number affected	Expected random pairs	Std. Error	Observed pairs
Prolif.	73	12	1.81	± 1.34	2
All galled plants	73	31	12.74	± 3.57	12
Galled and failed or prolif.	73	29	11.12	± 3.34	10

D. Incidence of galling and proliferation on stock from seedlings and on rose wilt virus (sensu Fry & Hammett) infected stock.

Thanks to the courtesy of Dr. Hammett of Plant Diseases Division Auckland the opportunity was given to assess the incidence of galling and proliferation in an experiment he had carried out at PDD.

Two rows of stock were planted out in winter 1969. One row comprised stock cuttings taken from selected seedlings of Rosa multiflora and accordingly presumed virus free. The second row comprised cuttings of Rosa multiflora which had been infected with rose wilt virus (sensu Fry & Hammett) by budding the plants with infected buds prior to the cuttings being taken.

The scion buds used in the experiment comprised 9 separate clones of Hybrid Teas which had been grown from seed and were accordingly also presumed free of virus.

The two rows of stock were divided into 9 plots each. The nine clones of seedling Hybrid Teas were budded with one clone on each of the nine plots on the stock from seedlings. The same nine clones of seedling Hybrid Teas were budded in the same sequence on correspondingly adjacent plots on the row of rose wilt virus infected stock. Due to failure of some of the stock not all plots contained the same number of stocks.

The plots were assessed with respect to the number

of buds which had grown normally; the number of buds which were proliferated and the number of buds which had failed. In each of the above categories those which showed galling symptoms were recorded.

These results are shown on Tables X, XI, & XII. It can be seen from the correlation of bud failure, and proliferation with galling that almost without exception those plants which were galled were those on which the buds had either failed or become proliferated.

The most notable result from this assessment is that the close correlation of the presence of galls with either bud failure or proliferation occurred both on the rose wilt virus infected stock and on the presumed virus free stock from seedlings, despite both having been budded with presumed virus free scions from seedling Hybrid Teas. In fact the number of buds which grew successfully into normal plants was significantly ($0.02 < P < 0.05$) greater on the rose wilt virus infected stocks than on the seedling stocks. Also the number of plants which showed gall symptoms was significantly ($0.02 < P < 0.05$) less on the rose wilt virus infected stocks than on the seedling stocks.

The above results are strong evidence against the assumption that bud failure and proliferation associated with galling are in any way separately or collectively related to the incidence of rose wilt virus.

Summary:

(1) The presence of galls are associated with the occurrence of proliferation and bud failure.

(2) Proliferation and bud failure associated with galling are not resultant on infection with Rose Wilt Virus.

Table X:

Clones of seedling Hybrid Teas	Budded on stock from seedlings								Budded on R.W.V. (sensu Fry & Hammett) infected stock							
	Number of buds which grew normally	Number with gall	Number of buds grew proliferated	Number with gall	Number of buds failed	Number with gall	Total with gall	Number budded	Number of buds which grew normally	Number with gall	Number of buds grew proliferated	Number with gall	Number of buds failed	Number with gall	Total with gall	Number budded
M	1	0	1	1	9	9	10	11	9	0	0	0	2	2	2	11
N	6	0	1	1	3	3	4	10	8	0	0	0	1	1	1	9
B	5	1	2	2	5	5	8	12	7	1	1	1	3	3	5	11
H	6	1	1	1	5	5	7	12	8	0	0	0	3	3	3	11
G	6	0	0	0	3	3	3	9	9	0	0	0	0	0	0	9
K	8	1	0	0	2	2	3	10	4	0	2	2	4	4	6	10
A	7	0	1	1	2	2	3	10	6	0	1	1	3	3	4	10
C	5	0	4	4	2	2	6	11	8	0	1	1	1	1	2	10
D	3	0	0	0	3	3	3	6	8	0	0	0	5	3	3	13
T	47	3	10	10	34	34	47	91	67	1	5	5	22	20	26	94

Table XI:

Correlation of bud failure and proliferation with galling.
Budded on stock from seedlings.

	Buds grew	Buds Prolif.	Buds failed	Totals
Galls present	3	10	34	47
Galls absent	44	0	0	44
Totals	47	10	34	91

Table XII:

Correlation of bud failure and proliferation with galling.
Budded on rose wilt virus infected stock

	Buds grew	Buds Prolif.	Buds failed	Totals
Galls present	1	5	20	26
Galls absent	66	0	2	68
Totals	67	5	22	94

E. Comparison of cutting grown stock from seedling material with commercial stock (considered infected) with respect to incidence of galling, proliferation, bud failure and vigour of growth.

During early spring 1970 an exchange of Rosa multiflora stock cuttings was made with Dr. Hammett of Plant Diseases Division, Auckland. A batch of 220 cuttings from mixed seedling clones was received from Auckland. These cuttings were of rather mixed diameters and ages.

A comparable batch of cuttings from seedlings were retained at PDD, Auckland.

At the same time approximately 500 cuttings of a clone known under the code letters AND, were selected using wood of approximately the same age, from upright shoots of similar diameter, length and vigour from near the top of the stock mother plants. Only the lowermost cutting on each shoot was used and it was taken with the proximal end just below the first node above a point 4 inches above the origin of the shoot. The cuttings were divided non-selectively into groups of 25. Ten of the groups, chosen using random numbers, were combined and sent to Auckland. The two batches at Auckland were planted together with a third batch of rose wilt virus infected cutting as described by Fry & Hammett (68). The analysis six months later of the incidence of excessive callus is shown in Fry & Hammett (68: Table I), where the clone AND is shown as "Levin stock".

At Levin the batch of PDD cuttings from seedling stocks and the batch of cuttings of AND stock were planted in an area adjacent to a block which had shown considerable galling the previous year. They were planted in four rows three feet apart, with 15 inches between cuttings in each row. Each row was divided into eleven blocks of ten plants in each, five from AND cuttings and five from PDD seedling cuttings. The cuttings from the two sources were planted alternately so that every second cutting was from the same source. The first cutting in each block in the first and third rows was from AND stock and the first cutting in each block in the second and fourth rows was from PDD seedling stock.

The reason for this apparently excessive randomisation was firstly to eliminate any soil variables which may occur in the blocks and secondly as one or other of the stock may have indicated some response to soil variables or a soil-borne pathogen or vector the pattern of distribution of such a factor could have been more easily determined. As will be seen the incidence of galling and proliferation was negligible for both stocks and no pattern of distribution of soil factors affecting galling or proliferation was apparent.

In February 1971 the total length of all the shoots on each plant was determined as a measure of the top growth vigour.

Then 22 of the 44 blocks were selected at random. The individual plants in these blocks were then lifted weighed to determine fresh weight and observations on

the condition of the bark, presence of galling, size of root system and condition of roots was recorded.

The remaining 22 blocks were budded in March 1971 with buds from a seedling Hybrid Tea (clone A) obtained from PDD, Auckland and due to its known origin and history presumed free of graft transmitted viruses.

The budded stocks were headed at the beginning of October 1971 and assessed for bud take at the end of October 1971.

Early January 1972 the scion tops were cut off and weighed. The point of excision of the stock top and the bud union was examined for galling on each plant.

The 220 stock plants which were lifted for examination in February 1971 showed very little evidence of galling. Only 12 of the plants or 5.4% showed positive galling and the galls themselves were relatively small. All of the 12 plants on which galling was recorded were the AND stock (10.91% galled). This result was obtained using the same stock batches as reported on by Fry & Hammett and in view of their results and the small number of affected stocks recorded there is little evidence of the association of galling with any one of the stocks used.

The assessment in January 1972 of the remaining 220 plants which had been budded to produce rose bushes of the Hybrid Tea clone A showed galling on 11 of the plants. Of these 11, 8 were of the AND stock clone and 3 were of the PDD stocks from cuttings of seedlings, (Table XIII). All the galling recorded on the budded plants occurred at the budding wound and at the point

of excision of the stock top whereas the galling recorded on the unbudded stock plants which were lifted and examined in February 1971 occurred at the base of the original stock cutting although there was no significant difference in the total number of galled plants in the two samplings.

It should be noted that on all the galled plants the buds either failed or proliferated (Table XIII). The occurrence of galling on both the stocks lifted and on the budded plants occurred completely at random through the whole area.

The bud take was exceptionally high compared with normal commercial takes in most New Zealand nurseries in recent years as only 9 buds failed to take out of 220 (4%) with a further 7 plants becoming proliferated.

The high bud take was unexpected as more than 20% of the stocks at time of budding had made relatively little growth from the original stock cutting and would not, in commercial production, be considered suitable for budding. The 5 bud failures that were not galled were on the smallest of these stocks and were obviously failures due to lack of vigour in the stocks and inadequate sap flow. At least part of the unexpectedly high bud take could be due to the vigour of the scion clone. However there is a close correlation of the incidence of galling in the unbudded stocks with the incidence of galling in the budded stocks and its association with bud failure and proliferation. If galling on the original stocks is the cause of proliferation and one of the causes of bud failure then the low

original incidence of galling in this case could have contributed to the high bud take.

The difference of vigour between the two stocks and the resultant size of the plants is of considerable interest in relation to the performance of the presumably virus infected commercial AND clone compared with the stock plants grown from presumed virus free cuttings of several mixed seedling clones.

Examination of the recorded values of the fresh weight of stocks, the fresh weight of scion tops and the total length of shoots per stock showed quite clearly that the values of the variates for single individuals are not normally distributed. Histograms (Fig. 1) illustrate these distributions quite clearly.

The inclusion of several different clones in the cuttings from seedlings from PDD, with no doubt different genetic potential for growth, is apparent in the broad spread in the values of the variates, with low relative frequencies particularly in the measurements of weight of stock. The multimodal shape of the distribution as a result of the inclusion of several clones is apparent.

The distribution of the values of the variates for the measurements on the AND stock shows the highest frequencies in the lowest values of the variates. This is more pronounced in the measurements of the stock than of the scion. This particular stock clone has been used for many years commercially and at least until recently has produced very vigorous plants.

These findings however would be in conformity with

the assumption that this clone has become infected with a virus which tends to restrict initial growth from the stock buds and such growth as occurs is thin and short compared with uninfected plants.

It has however been noted from observations that this weak initial growth tends to occur only in the first shoots arising from the buds on the previous seasons wood which comprises the cutting itself. Autumn growth arising from the current season's wood on plants which have become established is quite normal and vigorous.

The histogram of the weights of scion tops shows a considerable increase in size of plant on AHD stock relative to those of PDD seedling stocks and a more normal distribution of the values of the variates. This recovery would be in part due to the recovery in autumn growth of the stock, as noted above, together with growth during spring and early summer 1971 under the influence of the scion top rather than the stock (Tables XIV & XV).

In view of the values of the variates for single individuals being not normally distributed the means, standard deviations and significance for these three sets of measurements have been determined using the means of the 5 individuals of each stock in each of the blocks as the unit data thereby working with more normally distributed variates (Table XIV).

The performance of the two batches of stock was very significantly different in each of the three sets of measurements with a probability very much less than

0.001 (Table XV).

Despite the very great differences in stock growth prior to budding between the batch of cuttings from seedling stocks presumed free of virus and the commercial clone presumed infected with a virus the bud take appeared equally good on both batches with the following exceptions:- the five buds on stocks which had made practically no growth and were virtually unbuddable and the four buds which failed on galled stock.

It would appear therefore that the presence of such a presumed virus does not reduce bud take on normally buddable stocks although it may reduce the growth of some stocks to such an extent that they are unbuddable.

It was noted and recorded when lifting the unbudded stocks in February 1971 that almost without exception the plants of AND stocks had a considerable number of their first formed roots shrivelled, brown, and apparently dead. This symptom did not appear on any of the stocks from PDD seedlings even on the few that were smaller than many of the AND stocks. This symptom would therefore correlate with the AND stocks and possibly the presence of a virus rather than being a characteristic of smaller weaker plants.

Also noted at the same time was a roughness of the bark caused by small raised areas under the epidermis of the original stock cutting forming rounded lumps 1 to 2 mm in diameter purple in colour, becoming brown and dead as they age. This symptom correlated closely with stocks of smaller size and was not confined

to either source of stock.

Although the presence of dead roots appeared a characteristic of the presumably virus infected stock neither this nor the roughness of the bark was analysed further as they did not appear to have any bearing on the occurrence of galling or proliferation.

Summary:-

(1) A comparison was made between a commercial stock clone AND known to have shown extensive rose wilt virus symptoms (sensu Fry & Hammett), including proliferation and galling, with stock grown from cuttings taken from a mixture of selected clones of plants grown from seed and presumed free of rose wilt virus.

(2) There was no significant difference between the number of stocks showing galling at the base of the original cutting prior to budding and the number of stocks showing galling, 10 months after budding, at the point of budding and at the point of excision of the stock top.

(3) On all the budded plants which showed galling the buds either failed or became proliferated.

(4) No other plants showed proliferation symptoms and the only other bud failures (5 out of a total of 220) were due to unthrifty stocks without adequate sap flow.

(5) The presumably infected AND stock clone showed very significantly less growth and vigour than the presumed uninfected stock from seedlings.

(6) Despite the apparently "infected" condition of stock clone AND no bud failures occurred other than those explained in 3 and 4 above.

(7) An unusually low incidence of galling coincided with an unusually high bud take.

Table XIII:

Summary of galling, bud failure and proliferation on AND stock clone and PDD cuttings from seedlings budded with scions from a seed raised Hybrid Tea (clone A).

	Bud failed	Prolif.	Normal plants	Total
Galled AND	4	4	-	8
Galled PDD sdg.	-	3	-	3
Not galled AND	5	-	97	102
Not galled PDD sdg.	-	-	107	107
Total	9	7	204	220

Table XIV:

Comparison of stock vigour and weight of scion growth between AND stock clone and cuttings of seedling stocks from PDD.

Block means of 5 plants per block

	Shoot length (inches)		Stock weight (ounces)		Scion weight (ounces)	
Block	AND	PDDS	AND	PDDS	AND	PDDS
A 1	204	408			7.7	8.6
A 2	179	335			4.3	10
A 3	91	354	4.6	17.5		
A 4	99	322	4.2	17.5		
A 5	82	403			7.6	10.9
A 6	114	378			7.2	10.2
A 7	248	450	9.6	22.8		
A 8	107	380	5.6	21.3		
A 9	96	267	3.2	16.4		
A 10	114	279			6.4	15.4
A 11	105	384			6.8	10.1
B 1	92	298	3.9	14.1		
B 2	222	382			5.3	10.7
B 3	94	392	3.6	14.6		
B 4	65	465			5.2	12.4
B 5	31	145			4.8	9.4
B 6	112	320	4.1	10.4		
B 7	57	240			4.0	11.4
B 8	58	211	2.1	7.5		
B 9	140	357			6.0	10.7
B 10	124	431	4.7	14.7		
B 11	146	366	5.3	11.5		
C 1	120	449			5.5	10.5
C 2	28	227			3.2	9.8
C 3	37	247			5.1	4.7
C 4	20	202	0.9	8.4		
C 5	24	124			3.1	8.6
C 6	54	293			3.5	9.0
C 7	46	341	1.7	13.6		
C 8	32	217	1.3	6.2		
C 9	122	398			7.4	10.7
C 10	213	774	6.3	29.7		
C 11	61	396			3.6	13.9
D 1	31	353	not recorded			
D 2	107	262	"	"		
D 3	40	182	"	"		
D 4	39	190			3.6	6.0
D 5	23	359	1.2	13.5		
D 6	47	239	1.9	8.2		
D 7	154	449	4.4	21.2		
D 8	82	372			5.8	8.5
D 9	97	272			5.1	11.4
D 10	61	301			4.8	11.0
D 11	109	471	3.6	17.8		

Table XV:

Summary of comparison of stock vigour and weight of scion growth between AND stock clone and cuttings of seedling stocks from PDD.

	Stock	N	Mean	Standard Deviation	
Total length of shoots per plant, Feb. 1971 (inches)	AND	440	93.83	± 56.80	P < 0.001
	PDDS	440	333.72	± 112.27	
Fresh weight of stock plant Feb. 1971 (Ozs.)	AND	190	3.80	± 2.10	P < 0.001
	PDDS	190	15.08	± 5.96	
Fresh weight of scion growth Jan. 1972 (Ozs.)	AND	220	5.26	± 1.47	P < 0.001
	PDDS	220	10.16	± 2.28	

F. Analysis for evidence of grouping of plant failures and proliferation.

A large commercial block of rose stock cuttings was planted at the Avenue, Levin in May 1965. Four of the rows in this block were laid out as a trial to determine the effect of applications of potash, at three different rates, on bud losses and proliferation.

The three replicates were budded on 17/3/66, 21/3/66 and 6/4/66 respectively and were all budded by the same operator as was the fourth row which was budded on 17/3/66 and which was assessed as an extra control without potash application. The first replicate and the extra control were both budded with the cultivar 'Lady Seton'. The second and third replicates were budded with the cultivar 'Fragrant Cloud'.

The assessing and recording of the symptoms on each individual plant was carried out by Dr. Rainbow of Horticultural Research Laboratory, Levin. The plants were assessed on four occasions 27/9/66, 13/10/66, 9/11/66 and 12/12/66. The performance of these plants were recorded on each occasion with respect to the symptoms they were showing at the time of each recording. An analysis and report (160) has already been carried out with respect to the percentage of plants recorded in each of the categories, the changes in these percentages through the four recordings and the lack of any indication that potash has any effect on plant losses.

In the above report this trial was not analysed to determine if there was any evidence of grouping of the losses such as may be caused by spread of a pathogen or adjacent plants being affected in groups as would be expected from the use of budsticks which may carry a systemic disorder.

As this trial contained a considerably larger number of plants (2283) than had been assessed for grouping of losses in other trials (B, C, G, J) and as the occurrence of symptoms had been assessed independently, the testing of the data for grouping of losses would be expected to supply valuable further evidence. (See appendix B for method).

Each of the 12 plots and the extra control row were tested for grouping beyond that which would be expected from a random occurrence (Table XVI). The recording by Rainbow did not include any assessment of galling but did include some extra categories. Stunted was applied to dwarfed growth from the bud which frequently showed multiple shoot formation but which differed from typical proliferation in the shoots having greatly shortened internodes and being leafy to the base with the leaflets not conspicuously reduced in size but thickened clear veins. In the first instance these were included in the test for grouping as affected plants.

In view of the correlation shown in later observations between either proliferated or bud failed plants being galled both these categories were taken as being affected. Plants recorded as shoot dead

were also included as affected but most of these would have been included in any case as generally they occurred in the second, third and fourth recording as the result of failure of previously proliferated shoots.

Therefore plants shown as being affected in Table XVI, are those which at any of the four inspections showed any of the symptoms recorded as proliferated, stunted, bud failed or shoot dead. Blow outs are recorded according to the symptoms they showed before being blown out.

It can be seen that the only plots showing any evidence of the grouping of plants showing any of these symptoms were plot 4 and the extra control row. Further analysis was carried out to determine whether grouping was occurring in some of the symptoms.

Table XVII shows that this was so and that the stunting symptom shows significant grouping of affected plants. Omitting the stunting symptom from the aggregate of symptoms being tested gives a result which shows no evidence of grouping. Proliferation on its own without stunting shows no evidence of grouping of affected plants nor does bud failure and proliferation taken together without the stunting symptom show grouping.

The incidence of stunting in the other plots was generally so low that testing of the stunted plants for grouping did not give any meaningful results.

Summary:

(1) Bud failure, proliferation and plants on which the shoots have died are distributed at random along the row.

(2) There is no evidence of a pathogen causing all or any of the symptoms, bud failure, proliferation or dead shoots, spreading from primarily infected plants to adjacent uninfected plants.

(3) There is no evidence of all or any of the symptoms, bud failure, proliferation or dead shoots, occurring in groups due to transmission of systemic infection by sequential budding of a group of buds taken from a systemically infected budstick.

(4) The occurrence of the condition described by Rainbow as stunted is linked in groups in a pattern which suggests transmission systemically by buds from infected budsticks being budded in sequence.

Table XVI:

Test for grouping of affected plants in potash trial (Rainbow).

Note:- symptoms include all occurrence of proliferation, stunting, dead shoots, and bud failure taken collectively.

Plot number	Total plants	Number affected	Expected random pairs	Std. Error	Observed pairs	Evidence of grouping
1	118	65	35.25	± 5.94	38	-
2	94	55	31.60	± 5.62	29	-
3	134	92	62.48	± 7.90	66	-
4	146	60	24.25	± 4.92	33	+
5	149	71	33.36	± 5.78	39	-
6	150	101	67.33	± 8.21	73	-
7	187	114	68.89	± 8.30	75	-
8	162	75	34.26	± 5.85	38	-
9	173	135	104.57	± 10.23	111	-
10	154	103	68.22	± 8.26	71	-
11	148	74	36.5	± 6.04	42	-
12	148	132	116.84	± 10.81	119	-
Extra control	520	219	91.81	± 9.58	116	+

Table XVII:

Analysis for symptoms showing grouping in plot 4 and extra control (see Table XVI:).

	Symptom or symptoms assessed	Total plants	Number affected	Expected random pairs	Std. error	Observed pairs	Evidence of grouping
Plot 4.	Stunted	146	29	5.56	± 2.36	12	+
	Proliferated not stunted	146	21	2.88	± 1.70	2	-
	B.F., P., not stunted	146	28	5.18	± 2.28	5	-
	P., S.D., B.F not stunted	146	31	6.37	± 2.52	6	-
Extra control	Stunted	520	27	1.35	± 1.16	7	+
	Proliferated not stunted	520	133	33.76	± 5.81	36	-
	B.F., P., not stunted	520	165	52.04	± 7.21	56	-
	P., S.D., B.F not stunted	520	175	58.56	± 7.65	66	-

G. Analysis of observations on severely galled block of rose cultivar 'First Love'.

These observations were not made on a replicated experiment but were merely a detailed recording of forty plants in sequence in a row of the rose cultivar 'First Love'. The recording was made in May just prior to normal lifting of the plants for sale.

This block showed a high incidence of galling. The position of the plot of forty plants was chosen at random. The plants were lifted in order that the roots and proximal end of the original stock cutting could be examined. Details were recorded as to the growth or failure of the bud, (little proliferation remained at time of recording), the pattern of root formation (Figs. 16 & 17) and the presence or absence of galling at each of the following positions on the plant:- the roots, the proximal end of the original stock cutting, ground level, the wound at point of budding and the point of excision of the stock top (Fig. 18). The pattern of root formation was recorded with respect to distribution of the roots around the base of the original stock cutting as this appeared to be restricted by the presence of galls on the base of the stock cutting at time of root formation. Where cuttings were galled at the base and showed reduced distribution of roots around the base then the position of the roots invariably occurred on that part of the cutting which had the least galling (Figs. 16 & 17). If the roots spread in a complete

circle around the base of the original cutting this was taken as 100% root formation and recorded by the code number 4. If the roots only spread in $\frac{3}{4}$ of a circle with no root formation in the remaining $\frac{1}{4}$ this was recorded as 3. Similarly $\frac{1}{2}$ a circle was recorded as 2 and $\frac{1}{4}$ circle of roots was recorded as 1.

The results have been condensed in Table XX. For simplification in Table XVIII root galling has been omitted as it was only of occasional occurrence. Root galling only occurred when there was extensive galling elsewhere and only on plants on which the buds had failed. Basal galling is shown separately in order to test its relationship with root spread, but the presence of galling at ground level budding point and at the point of excision of the stock top have been combined and recorded as presence or absence of galling at ground level or above.

The recording of galling at ground level or above is comparable with the recording carried out on plots where the plants were not lifted.

It can be seen from the correlation of galling at ground level and above with bud failure, Table XIX, that all those stock on which the buds failed were galled. Of the buds which grew 4 also showed galling but 2 of these were also showing proliferation symptoms. Of the 16 which grew without showing galling at ground level or above 3 were showing proliferation symptoms but these 3 were the only 3 of the 16 which were galled at the base of the stock cutting.

This shows very significant correlation between the occurrence of galling and the failure of buds. Where proliferation occurred this correlated with the occurrence of galling, but it is probable that due to the elapse of time from initial bud growth to the time of recording that more proliferation may have occurred earlier in growth and had failed and been lost.

With respect to the correlation of root spread and galling at the base of the cutting the mean of the root spread indices on those plants without galling on the base of the cuttings is 3.5 compared with the mean of 2.25 on those plants with galling on the base of the cuttings. These means differ significantly ($P < 0.001$).

As the plants in these observations had been recorded in sequence along the rows further analysis was carried out to determine whether there was any linking in groups of affected plants beyond that which could be expected from a random distribution.

The following categories were tested for grouping of distribution of affected plants in the manner described in appendix B. Base of original stock cutting galled, galling at ground level and above ground, all plants showing any symptoms of galling at all irrespective of position of galling and the distribution of those plants on which the buds had failed. In all cases there was no significant departure from a random occurrence of affected plants.

Summary:

(1) The presence of galls are associated with bud

failure and the occurrence of proliferation.

(2) Galling at the base of the original stock cutting reduces the occurrence of roots. A gall occurring on one side of the base tends to restrict the occurrence of roots to the other side of the base.

(3) The occurrence of galled plants is at random along the row. There is no evidence of spread of galling from infected plants to adjacent plants.

(4) The occurrence of plants on which the buds have failed is at random along the row.

(5) There is no evidence of association of either galling or bud failure with transmission from infected scion sticks.

Table XVIII:

Correlation of galling, bud failure, and reduction of root spread in cultivar 'First Love'.

G-Bud grew: F-Bud failed: (P)-proliferated.
Roots 4-full circle: 3-$\frac{3}{4}$ circle: 2-$\frac{1}{2}$ circle: 1-$\frac{1}{4}$ circle.
+ galling present: - galling absent.

Plant	Bud	Roots	Cutt. base	Ground level up
1	F	1	+	+
2	F	4	-	+
3	G	4	-	-
4	F	3	+	+
5	F	2	+	+
6	F	3	+	+
7	G	4	-	-
8	G	3	-	-
9	G	3	-	-
10	G	4	-	-
11	F	2	+	+
12	G	4	-	-
13	F	2	+	+
14	G	3	-	-
15	G	4	-	-
16	F	1	+	+
17	F	3	-	+
18	F	4	+	+
19	G	3	-	-
20	G	4	-	-
21	G	3	-	-
22	G	4	-	-
23	F	3	+	+
24	G	2	+	+
25	G	2	+	+
26	F	4	+	+
27	F	4	+	+
28	F	1	+	+
29	G(P)	2	+	+
30	G(P)	3	+	-
31	G(P)	1	+	-
32	F	3	+	+
33	F	4	-	+
34	F	3	+	+
35	G(P)	1	+	+
36	F	1	+	+
37	G(P)	2	+	-
38	F	2	+	+
39	F	2	+	+
40	G	2	-	-

Table XIX:

Correlation of galling at ground level and above with bud failure.

	Buds failed	Buds grew	Total
Galling	20	4 (2P)	24
No galling	0	16 (3P)	16
Total	20	20	40

Table XX:

Correlation of root-spread and galling at the base of the cutting.

	No. of plants	Sum of root-spread indices	Mean root-spread index
Base of cutting galled	24	54	2.25
Base of cutting not galled	16	56	3.5

There is a significant correlation between the incidence of galling and reduction of the root-spread ($P < 0.001$).

Table XXI:

Test for grouping of affected plants.
Cultivar 'First Love'.

Types of Symptoms	Total plants	Number affected	Expected random pairs	Std. Error	Observed pairs
Base of cutting galled	40	24	13.8	± 3.71	16
Galling at ground level and above ground	40	24	13.8	± 3.71	16
Total of plants galled above or below ground	40	27	17.55	± 4.19	21
Buds failed	40	20	9.5	± 3.08	10

All types of symptoms tested occur at random in the row.

H. Occurrence of galling in rose stodk cuttings prior to budding.

A field of approximately 100,000 rose stock cuttings from a single clone were set out in May 1969 in rows 3 ft. apart with approximately 6 inches between the cuttings in the rows. These were planted in the course of ordinary nursery production by unskilled labour with no selection of the cutting for caliper or type. This is the field on which analysis appendix I: was subsequently carried out.

In order to ascertain whether galling was occurring in the stocks prior to the budding operation a small random sample of ten plots with ten cuttings in each was lifted in the last week in October 1969 (5 months after planting) and examined for the presence of galling at the base of the cutting.

The random location of the plots was determined using random numbers, the first half of the number determining the row counting from the Eastern end of the block and the second half of the number determining the number of yards along the row from the Northern end of the block. The first ten cuttings South along the row from the point thus determined were taken as the random sample. Recordings were made in sequence of the plants in each plot. The base of the cuttings were examined for galling and recorded. Amount of top growth was measured as length of each individual shoot. Leaf size and colour was assessed. Root types tended to fall into two categories, white

unbranched fleshy roots and superficially brown branched fibrous roots.

Heavily galled cuttings invariably only carried white unbranched fleshy roots but this type of root was also occasionally found mixed with brown branched fibrous roots on lightly galled and ungalled cuttings. It appeared that this difference was merely associated with root age and that root growth had been delayed on the heavily galled cuttings and was only of relatively recent origin (Figs. 13, 14 & 15).

Similarly heavily galled cuttings invariably had very much reduced stunted top growth, (Figs. 10, 11 & 12), although some cuttings had smaller tops of slightly different appearance and were probably slow in establishing for other reasons as they were not galled. By budding time, during December through to March, these differences in top growth were no longer apparent. Thus it would appear that although severe galling is invariably associated with delayed rooting and reduced stunted top growth in the early stages of cutting establishment delayed rooting and reduced top growth may also occur for other reasons.

The incidence of galling on the proximal end of the original stock cutting showed considerable variation between plots (Table XXII). It is uncertain whether this wide variation in the incidence of galling recorded is due to the small number of widely separated plots and the few plants per plot in relation to the size of the block or whether in fact the incidence of galling did vary in different parts of the block. It was

certain however that obvious galling did occur in some stock plants and not in others prior to the budding operation. The mean of the plants showing galling recorded on the plots assessed was 5.1 per plot with a standard deviation of ± 2.47.

Summary:

(1) Obvious galling occurred at the proximal end of the original stock cuttings on some plants and not on others in this block, prior to the budding operation.

(2) Galled plants invariably showed delayed rooting and reduced stunted top growth in the early stages of establishment although some stunting of slightly different appearance did also occur on some ungalled plants.

Table XXII:

Occurrence of galling on proximal end of rose stock cuttings prior to budding.

Plot No. and position	N.	Number of plants galled			
10 - 74	10	1			
12 - 68	10	4			
13 - 95	10	6			
24 - 18	10	9			
26 - 45	10	5	$\bar{x}$	=	5.1
30 - 02	10	8	s^2	=	6.1
36 - 81	10	4	s	=	2.4698
57 - 96	10	6			
59 - 86	10	2			
85 - 23	10	6			

I. Incidence of galling with scion growth and on stocks which were not budded.

A field of approximately 100,000 rose stock cuttings from a single clone were set out in May 1969. This was the field described in appendix H. By chance half a row was missed during the budding operation the following summer (January 1970). This was not discovered until after the stock tops had been removed in August 1970 from both the budded and the unbudded plants.

In January 1971 the condition of the individual stocks in the unbudded row was recorded together with the corresponding budded stocks in the two adjacent rows, one on either side and 3ft. away from the unbudded row. For purposes of analysis 200 corresponding and adjacent stocks in each of the 3 rows were grouped in plots of 10 plants (Tables XXIII & XXIV). These were analysed to obtain further information on the occurrence of galling on budded plants and its relationship to bud failure, and to determine whether the incidence of galling on plants on which the bud had failed was due solely to lack of a growing top or whether the occurrence of galling was predetermined prior to bud failure.

Using the chi square test (Tables XXVIII & XXIX) a very significant correlation was found between the stocks on which the buds had failed to grow into a bush being galled and those in which the buds had grown into a bush, being not galled.

In the case of the cultivar 'Erotica' 10 of the total buds which grew showed some galling but of those 10 there were 6 in which the shoots were conspicuously proliferated. None of those which grew without galling showed any signs of proliferated shoots.

There were 32 buds which failed without showing any galling on the remaining stock. If galling occurs as a result of bud failure there is no obvious explanation as to why these were not galled. If, on the other hand, as will be shown subsequently is most likely, galling is a cause of bud failure these 32 would represent bud failures due to other causes such as faulty technique.

The cultivar 'Irish Mist' had a significantly higher bud take than 'Erotica' ($P < 0.001$). Nine of the buds which grew showed some galling and two of these were proliferated. Of the bud failures only 5 were without galling, possibly a reflection of the higher bud take in this variety with consequently lesser failures from other causes.

There is no doubt from the above comments and the high degree of correlation shown in Tables XXVIII & XXIX that on those stocks which show galling the buds generally fail. Also proliferation is linked with galling in that all the proliferation observed on both 'Erotica' and 'Irish Mist' was associated with galling.

The problem is to decide whether the galling occurs as a result of bud failure and the consequent loss of actively growing shoots and leaves on the plant (reduction of shoots and leaves in the case of prolif-

erated plants) or whether bud failure occurs on those plants which are already predisposed to galling.

The row which was not budded throws considerable light on this question. If galling occurs as a result of there being no actively growing top on the plant then all those which were not budded but which were headed back in August 1970 at the same time as the budded plants, should tend to show galling.

The mean of galled unbudded plants was not significantly different ($P>0.10$) from the mean of galled plants which had been budded with the cultivar 'Erotica' (Table XXV) nor from the mean of galled plants which had been budded with the cultivar 'Irish Mist' (Table XXVI).

Although lack of a significant difference does not necessarily imply that there is no difference in the galling between the budded and the unbudded stocks it does provide positive evidence that the occurrence of galling is not caused entirely by lack of a growing top.

These three rows are part of the block recorded in appendix H where it was found that during the establishment period of the stock cutting some of the stocks were galled at the proximal end of the cutting and some were ungalled. The proportion affected with galling was not inconsistent with the occurrence of galling in these observations subsequent to budding.

On the other hand the presence of a growing top does appear to completely suppress the expression of the galling symptom at ground level and above. This is merely based on observations on this block and others

rather than recorded and statistically evaluated data, but the observations were made over several years on 4 to 5,000 stocks adjacent to or intermingled with budded and bud failed plants which showed varying incidence of galling and/or proliferation from very severe to slight occurrence. At no time were unheaded stocks with growing tops observed with galling at ground level or above.

Similarly it appears from frequent observations that certain strong growing, highly compatible scion varieties which regularly give a high percentage bud take may graft successfully on a proportion of the stocks which would potentially show excessive galling but because of the successful growth of the scion variety the symptom of excessive galling is suppressed.

These observations are not recorded in the hope of providing any real evidence in themselves but as a possible explantion why the mean of the galled plants budded with 'Irish Mist' is significantly ($P<0.02$) less than the mean of the galled plants budded with 'Erotica' (Table XVII). The cultivar 'Irish Mist' is a variety which usually has a high bud take and which appears by its growth to suppress the galling symptoms below the usual average for the area. Further evidence of this is present in appendix J. This is supported by the significantly higher ($P<0.001$) yield of plants of 'Irish Mist' than that of 'Erotica'.

Summary:

(1) There was very significant correlation between the occurrence of bud failure and galling.

(2) All the plants which showed proliferation symptoms were also galled.

(3) 'Irish Mist' had a significantly higher yield of plants ($P < 0.001$) and showed significantly less galling ($P < 0.02$).

(4) The incidence of galling on unbudded plants none of which had growing tops was not significantly different, neither from 'Erotica' ($P > 0.10$) nor from 'Irish Mist' ($P > 0.10$).

(5) The number of galled plants which had been budded with 'Irish Mist' was significantly less than the number of galled plants which had been budded with 'Erotica' ($P < 0.02$). It was suggested that this could be due to the higher successful bud take with the cultivar 'Irish Mist' suppressing some of the galling by successful scion top growth.

Table XXIII:

Effect of budding and scion growth on incidence of galling.
Cultivar 'Erotica' and unbudded stocks.

	Budded with cultivar 'Erotica'					Row not budded
Plot No.	Buds grew	With gall	Buds failed	With gall	Total galled	Number galled
1	2	0	8	5	5	5
2	3	0	7	5	5	4
3	3	0	7	6	6	4
4	5	0	5	4	4	5
5	5	3	5	4	7	1
6	7	2	3	1	3	4
7	4	0	6	5	5	4
8	5	0	5	3	3	5
9	6	2	4	2	4	4
10	8	0	2	0	0	3
11	6	1	4	4	5	1
12	3	0	7	6	6	4
13	1	0	9	7	7	3
14	6	1	4	2	3	3
15	4	0	6	5	5	2
16	5	1	5	2	3	3
17	4	0	6	4	4	4
18	7	0	3	2	2	4
19	5	0	5	4	4	8
20	4	0	6	4	4	4
T	93	10	107	75	85	75
$\bar{x}$	4.65	0.5	5.35	3.75	4.25	3.75
s^2	3.08	0.79	3.08	3.25	2.83	2.30
s	1.76	0.89	1.76	1.80	1.68	1.52

Table XXIV:

Effect of budding and scion growth on incidence of galling.
Cultivar 'Irish Mist' and unbudded stocks.

	Budded with cultivar 'Irish Mist'					Row not budded
Plot No.	Buds grew	With gall	Buds failed	With gall	Total galled	Number galled
1	4	0	6	6	6	5
2	8	0	2	2	2	4
3	5	1	5	5	6	4
4	7	0	3	3	3	5
5	5	0	5	3	3	1
6	10	0	0	0	0	4
7	4	0	6	6	6	4
8	6	0	4	4	4	5
9	9	0	1	1	1	4
10	4	0	6	6	6	3
11	10	0	0	0	0	1
12	9	1	1	0	1	4
13	10	2	0	0	2	3
14	8	2	2	2	4	3
15	9	0	1	1	1	2
16	7	1	3	3	4	3
17	9	0	1	1	1	4
18	7	1	3	3	4	4
19	9	0	1	1	1	8
20	7	1	3	1	2	4
T	147	9	53	48	57	75
$\bar{x}$	7.35	0.45	2.65	2.4	2.85	3.75
s^2	4.34	0.47	4.34	4.36	4.24	2.30
s	2.08	0.69	2.08	2.09	2.06	1.52

Table XXV:

Test for significance of difference of means of galled plants budded with 'Erotica' and galled plants not budded.

$s^2 = (43.75 + 53.75) \div 38$

$= 2.5658$

$d = 0.9872 \qquad n = 40 \qquad P > 0.10$

Table XXVI:

Test for significance of difference of means of galled plants budded with 'Irish Mist' and galled plants not budded.

$s^2 = (43.75 + 80.55) \div 38$

$= 3.2711$

$d = 1.5737 \qquad n = 40 \qquad P > 0.10$

Table XXVII:

Test for significance of difference of means of galled plants budded with 'Erotica' and galled plants budded with 'Irish Mist'.

$s^2 = (53.75 + 80.55) \div 38$

$= 3.5342$

$d = 2.3553 \qquad n = 40 \qquad P < 0.02$

Table XXVIII:

Data on correlation of bud failure and galling

Cultivar 'Erotica'.

	With gall	Without gall	Total
Buds failed	75	32	107
Buds grew	10	83	93
Total	85	115	200

Chi square = 69.2869

with one degree of freedom shows very significant correlation

Table XXIX:

Data on correlation of bud failure and galling

Cultivar 'Irish Mist'.

	With gall	Without gall	Total
Buds failed	48	5	53
Buds grew	9	138	147
Total	57	143	200

Chi square = 132.2032

with one degree of freedom there is very significant correlation.

J. Incidence of galling on stocks not budded, and on budded stocks with 100% take.

A short row was noticed in a commercial production block consisting of 42 stocks of which 30 stocks had not been budded and the remaining 12 stocks at one end of the row had been budded with 'Irish Mist' and all of the 12 buds had grown into normal plants. Despite the relatively small number of plants involved and the lack of replication this row was selected for lifting and recording in order to ascertain if there was any deviation from the pattern as shown in appendix G.

The plants were lifted and recorded for galling on roots, galling at base of original cutting, and galling at ground level, budding wound and point of excision of the stock top. A summary of this recording is shown in Table XXX.

It was noted that the galling on the unbudded stocks that had had their stock tops removed, generally showed symptoms at ground level and above on those plants which had below ground galling. On the other hand the budded plants, on which the buds had grown normally, only showed galling at the base of the original stock cutting. This observation would be in agreement with the apparent suppression of galling at ground level and above by the successful establishment and growth of a strong scion top as suggested in appendix I.

Despite there being no above ground symptoms on these plants galling was present at the base of the original cutting on approximately the same percentage

of plants as galling occurred on the unbudded plants (Table XXXI).

Due to the relatively small numbers of plants recorded and the lack of replication no real significance can be attached to these findings except that they confirm that initial galling can occur on budded plants which grow normally and apparently suppress the development of above ground symptoms.

It was also noted that, even on plants without any growing top, below ground galling occurred without any symptoms of galling necessarily being apparent at ground level or above. This also occurs if stock is headed back during the summer.

There was no evidence of association of galled plants other than would be expected from a random occurrence (Table XXXII).

Summary:

(1) There was no evidence of spread of galling from affected to unaffected plants.

(2) The occurrence of galling in relation to budded and unbudded plants supported findings in other field observations but due to lack of replication and the small number of plants observed no significant evidence can be claimed.

(3) Despite lack of above ground galling symptoms on plants with 100% bud take, galling at original cutting base indicated a similar original incidence, suggesting suppression of above ground symptoms by successful take, of a strong growing variety.

Table XXX:

Incidence and position of galling on row containing 30 unbudded stocks and 12 stocks budded with 'Irish Mist'.

	Plant number	Galling		
		stem base and roots	ground level and above	at any position
Stocks not budded but with stock tops excised	1	+	-	+
	2	-	-	-
	3	+	+	+
	4	+	+	+
	5	+	-	+
	6	+	+	+
	7	+	+	+
	8	+	-	+
	9	+	-	+
	10	+	+	+
	11	+	-	+
	12	+	-	+
	13	-	-	-
	14	-	-	-
	15	-	-	-
	16	-	+	+
	17	+	-	+
	18	-	-	-
	19	+	-	+
	20	+	-	+
	21	+	+	+
	22	+	-	+
	23	-	+	+
	24	+	-	+
	25	+	+	+
	26	-	-	-
	27	-	-	-
	28	+	+	+
	29	+	-	+
	30	-	-	-
Budded and grew	31	+	-	+
	32	+	-	+
	33	-	-	-
	34	+	-	+
	35	+	-	+
	36	+	-	+
	37	+	-	+
	38	+	-	+
	39	-	-	-
	40	+	-	+
	41	+	-	+
	42	-	-	-

Table XXXI:

Comparison of number of galled plants of unbudded stocks and budded stocks (all buds grew) Cultivar 'Irish Mist'.

	Not budded		Budded	
	Number	% of total	Number	% of total
Galled	22	73.33	9	75
Not galled	8	26.66	3	25
	30	100	12	100

Table XXXII:

Test for association of galled plants of unbudded stocks and budded with 'Irish Mist'.

	Total plants	Number affected	Expected random pairs	Std. error	Observed pairs
Galled not budded	30	22	15.4	± 3.92	17
Galled budded	12	9	6	± 2.45	6
Total stocks galled	42	31	22.14	± 4.71	23

K. Correlation of stock top symptoms of galling with subsequent bud failure and proliferation on the same stocks.

In the hope of avoiding various viruses proliferation and bud failure a clone of cutting grown Rosa multiflora stocks was built up from an original selected seedling under carefully controlled conditions to avoid mechanical and graft transmitted infection of the stock. This clone is referred to as SS stock.

During the build up period two rows of this stock were planted in late May 1970 for testing with imported budwood.

At the end of October 1970 at the same time as the assessments in appendix H were being carried out 5 plots of 5 stocks each were selected at random in the two rows of SS stock. These were lifted and examined for basal galling (Table XXXIII).

During the lifting of the stocks for these observations and from numerous lifting of stocks for bacterial isolations and spot checks of field crops it was found that almost without exception it became possible to recognise, from the appearance of the growth of the stock top at this stage, which plants were galled and which were not. No one measurable or discernable characteristic could be correlated with the occurrence of galling but rather a combination of slight differences in leaf colour, size, shape, degree of rolling of leaflets internode length at base of shoots, shoot thickness and a tendency towards rough corky swelling

with protruding nodules occurring in the region of the scars left by the bud scales from which the shoot emerges (Figs. 10, 11 & 12).

As the degree of galling varies so does the ease with which the signs on the top growth can be discerned as they also appear to vary in degree.

Accordingly the five plants on either side of the plots lifted were assessed from the appearance of their top growth as to their being galled, possibly galled and normal.

These plants were budded in November 1970 with scion buds imported from Europe. After heading back the stock tops the scion buds started into growth and were recorded for bud failure, proliferation and normal growth. This shows a close correlation with the estimation of galling from the appearance of the stock tops prior to budding (Table XXIV).

Despite the relatively small number of plants assessed the correlation between the symptoms observed on the stock tops and the subsequent failure or proliferation of the buds is highly significant with a probability of occurring by chance of considerably less than 0.001.

It would be tempting to infer from the results (Table XXIV) that the plants showing less distinct top symptoms and presumed lightly galled are those which tended to proliferate, whereas those heavily galled tended to fail, but it would be unwise to make this assumption without further trials and further evidence as to the reliability of the method of assessing galling.

There is no doubt however that proliferation can occur on stock taken from a seedling and budded with imported budwood which can be presumed free of graft transmitted viruses having been raised from seedling cultivars and only budded on seedling stocks.

Summary:

(1) Galling occurs on stocks grown from cuttings taken from a seedling R. multiflora.

(2) Symptoms can be recognised with practice on the tops of young cutting grown stocks on those plants which are predisposed to show bud failure or proliferation after budding.

(3) Proliferation occurs despite stock and scion material which has come from seedlings and never been in graft contact with any roses other than seedlings.

Table XXXIII:

Incidence of galling at the proximal end of the original cutting on stock plants grown from a selected seedling.

Plot No.	n.	Number galled per plot
1	5	1
2	5	1
3	5	1
4	5	3
5	5	5
Total	25	11

incidence of galling 44%.

Table XXXIV:

Correlation of top growth symptoms indicating galling prior to budding with subsequent growth of buds.

E.G. - Estimated galling from appearance of top growth
G. - Galled obvious symptoms.
(G) - Probably lightly galled, some symptoms
N. - Normal no symptoms.

B.T. - Bud take
F. - Bud failed
P. - Bud proliferated
N. - Bud growth normal.

Plot No.	E.G.	B.T.
1A	N	N
	N	N
	N	N
	G	F
	N	N
1B	N	N
	(G)	P
	(G)	F
	N	N
	N	N
2A	N	N
	N	N
	N	N
	N	N
	N	N
2B	N	N
	N	N
	(G)	P
	N	N
	N	N
3A	N	N
	N	N
	N	F
	(G)	N
	G	F
3B	(G)	N
	G	F
	(G)	F
	N	N
	N	N

Plot No.	E.G.	B.T.
4A	N	N
	N	N
	N	N
	N	N
	N	N
4B	N	N
	G	F
	(G)	P
	(G)	P
	(G)	F
5A	(G)	F
	(G)	F
	G	F
	G	F
	(G)	P
5B	(G)	P
	(G)	F
	G	F
	(G)	P
	(G)	F

Summary:

E.G.	Bud take F	Bud take P	Bud take N	Total
G	7	0	0	7
(G)	7	7	2	16
N	1	0	26	27
Total	15	7	28	50

G + (G) - 46%
F + P - 44%

P < 0.001

L. New Zealand Nurserymens Association, rose stock trials.

A growers trial was carried out by members of the New Zealand Nurserymen's Association in an endeavour to ascertain whether there were any striking differences in the production performance of the main stocks in common use in nurseries throughout New Zealand.

This Trial was carried out by the growers themselves and each individual reporting nursery recorded its own results. The individual recording by each reporting nursery together with variation in locality, climatic and cultural conditions between the various reporting nurseries would be expected to cause considerable variation between the results, and this was so, as will be seen later. On the other hand, if some of the stock clones carried any inherent or systemic defect it would be expected that this would become apparent as a significant decline in nursery productivity in such stocks.

Method:

Sixteen of the major rose producing nurseries in New Zealand agreed to supply cuttings of the strain of rosa multiflora stock which they normally use in their nursery production to each of the recording nurseries. In addition, the Horticultural Research Centre at Levin supplied two stocks under the code numbers A 69 and B 69 making stocks from eighteen different sources all told. These stocks were sent to each of the trial nurseries on the 30th April 1969. The stock clones were planted in random sequence in two rows with twelve

stocks from each stock source in the first row and twelve stocks from the same source adjacent in the second row.

During January/February 1970 the testing nurseries budded the stocks, the first row being budded with the cultivar 'Lady Seton' a reputedly high yielding easily budded variety. The second row was budded in January/February with the cultivar 'Virgo' a variety considered difficult to bud and which normally gives a low percentage yield.

The yield from the plots and the cause of failure were assessed in autumn 1971. That is, they were assessed at the time just prior to when the plants would normally become saleable. The bushes produced were classified as "weak" for those that would be below saleable standard, "average" for ordinary saleable grade and "strong" for any exceptionally vigorous plants. At the same time, those stocks which failed to produce bushes were differentiated into those in which the stock failed to grow and those in which the bud failed to take. In other words, if the stock died before it was possible to bud it, this was recorded as a different cause of failure to those in which the stock grew and was budded but the bud subsequently failed.

An attempt was made to record the amount of callus and galling at the point at which the stock was headed off but the different reporters varied considerably in their interpretation of this and the results are not comparable between recording nurseries.

Recording of the incidence of proliferation at time

of bud shooting was not sufficiently co-ordinated between the reporting nurseries to show anything of consequence in the pattern of occurrence and at the principal recording time in the autumn most of the plants that had shown symptoms would have either failed or grown out of it.

Source of stock and locality of trials.

Both the nurseries which served as the sources of stock clones and the reporting nurseries covered as wide a range of locality and climatic conditions as possible. The source of stocks and their locality were as follows:-

A. Walker, Hastings.

B. Donaldson, Dunedin.

C. Emson, Auckland.

D. Allenton, Ashburton.

E. Wallis, Dunedin.

F. Palmer, Auckland.

G. McKinnon, Cambridge.

H. Odering, Christchurch.

I. Avenue, Levin.

J. Mason, Feilding.

K. Matthews, Wanganui.

L. Blumhart, Whangarei.

M. Duncan and Davies, New Plymouth.

N. Seccombe, New Plymouth.

O. Kingsdown, Timaru.

P. Horticultural Research Centre, Levin.

Q. Bell, Auckland.

R. Horticultural Research Centre, Levin.

The reporting nurseries were located as follows:-

1. Duncan and Davies, New Plymouth.
2. Palmer, Auckland.
3. Walker, Hastings.
4. Wallis, Dunedin.
5. Seccombe, New Plymouth.

Analysis of yield of saleable bushes:

In order to look at yield of saleable bushes on each of the stock clones used, the resultant number of rose plants recorded as either "average" or "strong" were combined for both cultivars on each stock at each recording nursery. The number recorded is therefore the number of saleable bushes produced from 24 stocks of each stock clone at each reporting nursery.

In order to separate variations occurring in reporting nurseries from variations in stock clones, the results were analysed as a randomised block layout by treating each reporting nursery as a block with each stock clone a treatment within that block. The results of this analysis are shown in Table XXXV.

From this table it will be seen from the analysis of variance that there are significant differences both between the different stocks and between the reporting nurseries at the 1% level (Table XXXVI).

As far as the stocks are concerned the analysis showed that the least significant difference between the means at the 5% level of significance was 4.56, indicating that many of the stocks are not significantly different.

The stocks are arranged in order of their means and

it can be seen that there is no significant difference between stocks A to H, nor is there any significant difference between stocks B to K, or C to M and so on. This could be interpreted to mean either that there are insufficient blocks tested to give a significant result or that there is, in fact, little significant difference between the majority of the stocks tested. As there is 68 degrees of freedom in the residual variation it would seem that there are only relatively slight differences between stocks, particularly in the upper two thirds of the table from A to M. No stock clone or clones stand out as being notably better than the others but the differences are a continuous variation of degree rather than an empirical plus or minus difference such as one would expect if there were a clone or clones entirely infected with virus that affected yield of saleable bushes or a clone or clones without virus.

The considerable variation within each stock clone may indicate some random cause of failure not dependent either on the clone or on the variation between the blocks. In other words, many of the failures would appear to be caused by some factor which is neither completely inherent nor completely systemic in the stock clones tested, but which occurs at random with some predisposition towards certain reporting nurseries and certain stock clones. A varying degree of virus infection or symptom expression through the clones could cause this effect.

Analysis of stock failures:

In order to test at what stage most of the failures

occurred the trials were analysed for variance again on randomised block pattern to see if there was any difference between the various stock clones with respect to transplanting failure. See analysis of stock failure Tables XXXVII & XXXVIII.

It can be seen from the analysis of variance in this table that there was no significant difference between the trial stocks at the 5% level but that there were significant differences between the reporting nurseries at less than the 1% level. This would indicate that most of the variation in the actual successful take of the stock cuttings was dependent not on the stock clones but on the cultural practices and locality of the testing nurseries. Stock Q does show a widely divergent result from one of the reporting nurseries but as this one report differs so markedly from the others it is probably due to some special accidental circumstance.

Analysis of weak plants:

In order to determine whether the incidence of weak unsaleable plants had any significant influence on the yield of saleable bushes from the stock clones the trials were analysed for variance on a randomised block pattern comparing the number of weak plants per stock clone and per reporting nursery. See analysis of weak plants Tables XXIX & XL.

There was no significant difference between the stock clones at the 5% level, but the difference between the reporting nurseries were very significant at the 1% level. This could be a reflection of variation

of cultural practices and locality of the reporting nurseries, but is probably due to a lack of standardisation of recording what constitutes a weak plant as this would be likely to vary from one reporting nursery to another. On the other hand if any of the stock clones were consistently producing a greater number of weaker plants this would have been recorded and shown as a significant difference between stock clones. Stock M and Stock Q both show a single widely divergent result from one of the reporting nurseries, but these are probably due to some special chance circumstance.

Analysis of bud failures:

The principal cause of loss was due to bud failures, there being a total of 508 bud failures as compared with 194 stock failures and 186 weak plants. An analysis of bud failures (Tables XLI & XLII) showed significant differences at the 1% level in both the reporting nurseries and the stock clones. As the number of stocks available to bud in many of the plots was reduced by the stock failures the net number of bud failures was not a true expression of the bud failure rate. Accordingly a further analysis was carried out expressing bud failures as a percentage of actual number of stocks budded (Tables XLIII & XLIV). This is still only an approximation as there is considerable variation between the plots in number of stock that failed and this introduces some differential weighting of the bud failures expressed as a percentage of the number of surviving stocks per plot at budding time. It is however a closer approximation than table XLI & XLII.

Again both reporting nurseries and stock clones showed significant differences at the 1% level but with a reduced variance ratio for the reporting nurseries and an increased variance ratio for the stock clones.

As the complete failure of stock Q to grow in Block III seemed anomolous in the light of the performance of this stock in the other blocks, the missing plot method was used to provide an estimated figure for this plot.

Stocks P and R gave a considerably poorer bud take than the rest of the stocks, but apart from these two stocks, none of the remaining sixteen clones appear to be strikingly different from the others. Of these sixteen stocks the clones F, G, H, J, L and M are neither significantly different from clone A which has the lowest percentage bud failures nor significantly different from stock Q which has the highest percentage bud failures of the sixteen.

Comparison of yield of saleable bushes of the rose cultivars 'Lady Seton' and 'Virgo'.

In the nursery industry it is generally considered that 'Lady Seton' is an easily propagated high yielding variety and that 'Virgo' is more difficult to propagate with a lower yield of saleable bushes. For this reason these two varieties were chosen for the stock trials in the hope that at least one cultivar would accentuate any stock differences.

On analysis it was found contrary to expectations that there was no significant difference ($P > 0.10$) between the yields from the two cultivars.

Summary:

(1) From all the aspects analysed the reporting nurseries showed significant differences at the 1% level as was to be expected with widely differing localities, soils etc. and with a different person doing the recording at each nursery. There was no significant difference between the two cultivar clones used as test varieties.

(2) There was no significant difference at the 5% level between the stock clones in the number of stock cuttings which failed to grow, nor in the number of weak plants produced. However with respect to yield of saleable plants and number of buds that failed to take there were significant differences between the clones at the 1% level.

(3) Apart from clones P and R which were considerably lower in bud take the remaining sixteen clones appeared to differ by some random factor rather than any inherent major difference in the clones themselves.

Therefore if there is a virus or other systemic factor commonly present in commercial stock clones it is either present in all the clones tested possibly in varying titre or if only present in some clones it does not greatly affect bud take or yield of saleable bushes.

Table XXXV:

Reported plot yields of saleable plants.

Stock Clones	Randomised blocks I	II	III	IV	V	T_s	$\bar{x}_s$	s_s
A	18	21	21	16	21	97	19.4	2.30
B	15	22	21	19	17	94	18.8	2.86
C	22	14	19	17	16	88	17.6	3.05
D	18	18	13	16	21	86	17.2	2.95
E	13	21	17	12	19	82	16.4	3.85
F	20	20	10	16	12	78	15.6	4.60
G	13	16	17	16	14	76	15.2	1.64
H	16	20	17	13	9	75	15.0	4.18
I	16	15	15	18	9	73	14.6	3.36
J	20	17	11	10	15	73	14.6	4.17
K	22	15	8	14	13	72	14.4	5.03
L	16	19	11	10	13	69	13.8	3.70
M	13	17	14	9	15	68	13.6	2.96
N	12	19	13	9	3	56	11.2	5.85
O	14	11	7	8	14	54	10.8	3.27
P	12	13	14	4	9	52	10.4	4.40
Q	18	11	0	6	10	45	9.0	6.60
R	17	6	3	0	8	34	6.8	6.45
T_b	295	295	231	213	238			
$\bar{x}_b$	16.39	16.39	12.83	11.83	13.22			
s_b	3.24	4.23	5.76	5.23	4.73			

Total of all plots 1272

Mean of all plots 14.13

Table XXXVI:

Analysis of variance of yields of saleable plants

Source of variation	Sum of squares	Degrees of freedom	Mean square	F Variance ratio	P
Trial stocks	974	17	57.294	4.241	P <0.01
Reporting nurseries	323.7	4	80.9	5.988	P <0.01
Residual	918.6	68	13.5088	-	-
Totals	2216.3	89	-	-	-

		Stocks		Nurseries (Blocks)
Std. error of means	$\bar{x}_s$	± 1.6437	$\bar{x}_b$	± 0.8663
Std. error of difference between means		2.3245		1.2251
Confidence limits of means P0.05	$\bar{x}_s$	± 3.2217	$\bar{x}_b$	± 1.6979
Least significant difference between means P0.05		4.5561		2.4012

Table XXXVII:

Reported numbers of stock failures

Stock clones	Randomised blocks I	II	III	IV	V	T_s	$\bar{x}_s$	s_s
A	4	0	1	1	1	7	1.4	1.52
B	6	0	2	2	0	10	2.0	2.45
C	0	1	3	2	0	6	1.2	1.30
D	1	0	4	0	0	5	1.0	1.73
E	8	0	1	3	1	13	2.6	3.21
F	0	1	1	0	1	3	0.6	0.55
G	8	0	2	0	1	11	2.2	3.35
H	6	0	1	0	1	8	1.6	2.51
I	1	0	0	2	1	4	0.8	0.84
J	3	0	4	2	2	11	2.2	1.48
K	1	1	4	0	1	7	1.4	1.52
L	5	0	6	0	9	20	4.0	3.94
M	0	0	2	5	1	8	1.6	2.07
N	6	1	9	0	0	16	3.2	4.09
O	6	0	8	0	2	16	3.2	3.63
P	0	0	3	5	0	8	1.6	2.30
Q	1	0	24	2	2	29	5.8	10.21
R	0	0	9	2	1	12	2.4	3.78
T_b	56	4	84	26	24			
$\bar{x}_b$	3.11	0.22	4.67	1.44	1.33			
s_b	2.99	0.43	5.57	1.65	2.03			

Total of all plots - 194

Mean of all plots - 2.16

Table XXXVIII:

Analysis of variance of stock failures.

Source of variation	Sum of squares	Degree of freedom	Mean Square	F Variance ratio	P
Trial stocks	138.622	17	8.154	1.1916	$P > 0.05$
Reporting nurseries	218.489	4	54.622	5.622	$P < 0.01$
Residual	660.711	68	9.716	-	-
	1017.822	89	-	-	-

		Stocks		Nurseries (Blocks)
Std. error of means	$\bar{x}_s$	$\pm$ 1.3939	$\bar{x}_b$	$\pm$ 0.7140
Std. error of difference between means		1.9713		1.0097
Confidence limits of means P.0.05	$\bar{x}_s$	$\pm$ 2.7320	$\bar{x}_b$	$\pm$ 1.3994
Least significant difference between means P.0.05		3.8637		1.9790

Table XXXIX:

Reported numbers of weak plants

Stock clones	Randomised blocks I	II	III	IV	V	T_s	$\bar{x}_s$	s_s
A	2	2	0	4	0	8	1.6	1.67
B	3	1	0	0	0	4	0.8	1.30
C	1	0	0	5	5	11	2.2	2.59
D	5	0	2	4	1	12	2.4	2.07
E	2	0	1	4	2	9	1.8	1.48
F	1	1	5	5	2	14	2.8	2.05
G	1	1	2	4	0	8	1.6	1.52
H	2	2	0	8	0	12	2.4	3.29
I	3	0	4	3	3	13	2.6	1.52
J	0	1	5	7	3	16	3.2	2.86
K	0	0	3	2	2	7	1.4	1.34
L	1	1	4	2	0	8	1.6	1.57
M	10	1	3	4	1	19	3.8	3.70
N	2	3	0	4	0	9	1.8	1.79
O	2	2	3	4	3	14	2.8	0.84
P	1	1	0	0	0	2	0.4	0.55
Q	0	1	0	12	0	13	2.6	5.27
R	1	1	0	1	4	7	1.4	1.52
T_b	37	18	32	73	26			
$\bar{x}_b$	2.06	1.00	1.78	4.06	1.45			
s_b	2.34	0.84	1.90	2.88	1.62			

Total of all plots - 186

Mean of all plots - 2.07

Table XL:

Analysis of variance of weak plants

Source of variation	Sum of squares	Degrees of freedom	Mean Square	F Variance ratio	P
Trial stocks	61.2	17	3.6	1.1856	$P > 0.05$
Reporting nurseries (blocks)	100.1556	4	25.0389	5.8662	$P < 0.01$
Residual	290.2444	68	4.2683	-	-
Totals	451.6000	89	-	-	-

	Stocks		Nurseries (Blocks)	
Std. error of means	$\bar{x}_s \pm$	0.9239	$\bar{x}_b \pm$	0.4869
Std. error of difference between means		1.3066		0.6886
Confidence limits of means P.0.05	$\bar{x}_s \pm$	1.8108	$\bar{x}_b \pm$	0.9543
Least significant difference between means P.0.05		2.5609		1.3497

Table XLI:

Reported numbers of bud failures

Stock clones	I	II	III	IV	V	T_s	$\bar{x}_s$	s_s
A	0	1	2	3	2	8	1.6	1.14
B	0	1	1	3	7	12	2.4	2.79
C	1	9	2	0	3	15	3.0	3.54
D	0	6	5	4	2	17	3.4	2.41
E	1	3	5	5	2	16	3.2	1.79
F	3	2	8	3	9	25	5.0	3.24
G	2	7	3	4	9	25	5.0	2.92
H	0	2	6	3	14	25	5.0	5.48
I	4	9	5	1	11	30	6.0	4.00
J	1	6	4	5	4	20	4.0	1.87
K	1	8	9	8	8	34	6.8	3.27
L	2	4	3	12	2	23	4.6	4.22
M	1	6	5	6	7	25	5.0	2.35
N	4	1	2	11	21	39	7.8	8.35
O	2	11	6	12	5	36	7.2	4.21
P	11	10	7	15	15	58	11.6	3.44
Q	5	12	0	4	12	33	6.6	5.27
R	6	17	12	21	11	67	13.4	5.77
T_b	44	115	85	120	144			
$\bar{x}_b$	2.44	6.39	4.72	6.67	8.0			
s_b	2.79	4.46	3.03	5.48	5.35			

Total of all plots - 508

Mean of all plots - 5.64

Table XLII:

Analysis of variance of numbers of bud failures

Source of variation	Sum of squares	Degrees of freedom	Mean Square	F Variance ratio	P
Trial stocks	777.0222	17	45.7072	3.6769	P <0.01
Reporting nurseries (blocks)	328.2889	4	82.0722	6.6022	P <0.01
Residual	845.3111	68	12.4310	-	-
Totals	1950.6222	89	-	-	-

	Stocks	Nurseries (blocks)
Std. error of means	$\bar{x}_s \pm$ 1.5767	$\bar{x}_b \pm$ 0.8310
Std. error of difference between means	2.2298	1.1752
Confidence limits of means P.0.05	$\bar{x}_s \pm$ 3.0903	$\bar{x}_b \pm$ 1.6288
Least significant difference between means P.0.05	4.3704	2.3034

Table XLIII:

Bud failures as % of stock budded

(adjusted for stocks which failed to grow and for plot failure Block III Stock Q).

Stock Clones	Randomised blocks I	II	III	IV	V	T_s	$\bar{x}_s$	s_s
A	0	4	9	13	9	35	6.92	4.98
B	0	4	5	14	29	52	10.31	11.66
C	4	39	10	0	13	65	13.06	15.35
D	0	25	25	17	8	75	15	10.87
E	6	13	22	24	9	73	14.60	7.82
F	13	9	35	13	39	108	21.52	14.26
G	13	29	14	17	39	111	22.22	11.54
H	0	8	26	13	61	108	21.56	23.92
I	17	38	21	5	48	128	25.62	17.10
J	5	25	20	23	18	91	18.13	7.92
K	4	35	45	33	35	152	30.45	15.32
L	11	17	17	50	13	107	21.44	16.17
M	4	25	23	32	30	114	22.78	11.04
N	22	4	13	46	88	173	34.65	33.34
O	11	46	38	30	23	167	33.43	16.26
P	46	42	33	79	63	262	52.46	18.23
Q	21	50	(37)	18	55	181	36.20	16.29
R	25	71	80	95	48	319	63.82	27.72
T_b	203	483	470	539	626			
$\bar{x}_b$	11.25	26.82	26.11	29.97	34.79			
s_b	11.83	18.64	17.45	25.49	22.34			

Table XLIV:

Analysis of variance of bud failures as % of stocks budded.

Source of variation	Sum of squares	Degrees of freedom	Mean square	F Variance ratio	P
Trial stocks	17796.04	17	1046.83	4.62	$P < 0.01$
Reporting nurseries (blocks)	5597.53	4	1399.38	6.17	$P < 0.01$
Residual	15197.08	67	226.82	-	-
Totals	38590.65	88	-	-	-

Std. error of means	$\bar{x}_s \pm 6.7352$	$\bar{x}_b \pm 3.5498$
Std. error of difference between means	9.5249	5.0201
Confidence limits of means P.0.05	$\bar{x}_s \pm 13.2010$	$\bar{x}_b \pm 6.9576$
Least significant difference between means P.0.05	18.6688	9.8394

M. Bacterial isolation and transmission attempts.

Methods:

A number of different isolation methods were regularly used.

Galled material was prepared from actively growing galls by one of two methods:- 1. A small nodule 1 to 2 mm across was excised from the gall and surface sterilised in either 1% sodium hypochlorite solution or 0.1% mercuric chloride solution followed by rinsing in three changes of sterile distilled water. 2. Larger pieces of gall were carefully trimmed, using a freshly sterilised scalpel after each cut, to remove all the originally exposed surfaces and any surfaces which had probably been contaminated by the first cuts.

On each attempt to isolate a casual bacteria 10 to 20 separate pieces of galled material was used and each piece treated separately apart from surface sterilisation.

When each piece of gall was to be crushed in liquid, 5 mls was first sterilised in 19 x 150 mm test tubes each with an oxoid cap, cooled, the tissue inserted sterilely and crushed with a sterile glass rod.

All mediums used were sterilised by autoclaving for 15 minutes at 15 lbs. pressure (121°C). Petri dishes were sterilised in an oven for 1 hour at 150°C. Scalpels and forceps were sterilised in 95% ethyl alcohol and flamed before use. Normal laborabotry practice was followed in flaming platinum loops, mouths of tubes, sterilising benches etc.

Apart from special isolation media, culturing was carried out on, either Difco Nutrient Agar 23 grams/ 1000 mls and adjusted if necessary to pH. 6.8 or on Potato Dextrose Agar 39 grams/1000 mls adjusted to pH 6.5. Agar plates were poured immediately after autoclaving the medium, cured for 2 or 3 days before innoculating and incubating in an inverted position. Agar slants were autoclaved in the tubes which were 19 x 150 mm Pyrex rimless with oxoid caps.

Incubation was generally at 27°C although some batches were tried at 25°C and at 30°C.

Agar plates were invariably streaked three times to ensure some at least of the colonies being isolated. The first streaking was done close to the edge, the loop flamed and a second streaking, at right angles to the first and close to the edge of the plate, was done in such a way as to pick up and spread some bacteria from the edge of the first streak. The third streaking, picked up from the edge of the second and spread backwards and forwards over the rest of the plate. This method gave very good results in producing well isolated colonies.

All methods of isolation were directed towards isolating a causal organism from the galling and on account of the appearance of the galls being typical of crown gall they were methods that have been used successfully in isolating Agrobacterium spp. by other workers.

Direct Isolation.

A surface sterilised or surface trimmed piece of

gall tissue was cut in half with a sterile scalpel and the freshly cut surface used to streak an agar plate. Alternatively the piece of gall tissue was crushed in sterile distilled water as above and immediately streaked with a platinum loop.

Both these methods of direct isolation were aimed at obtaining isolations of micro-organisms as they occurred in the galls before any build up of saprophytes had changed the balance of population.

Patels medium:

A selective medium prepared after the method described by Patel (156) using 3 grams sodium taurocholate, 10 grams peptone and 15 grams of agar, with the addition of 20 grams dextrose and 2 ml of 1 in 1000 aqueous solution of crystal violet per 1000 ml distilled water.

A modification of this medium was also tried using 3 grams of bile salts, either Difco bile salts No. 3 or Oxoid bile salts, in place of the sodium taurocholate.

According to Patel the selectivity of this medium is due to suppression of coccus forms of bacteria by the bile component, inhibition of gram positive bacteria by crystal violet and suppression of psychrophilic soil bacteria by incubation at high temperature 27° to 30°C.

This medium was used for streaking rather than shaking the suspension of macerated gall with melted agar before pouring as described by Patel.

Streaking was carried out using a suspension of macerated gall in sterile water which had been stood

for 3, 6, 9 and 12 hours, following the recommendation of Patel to stand the suspension for 2 to 12 hours.

Also streaking immediately after maceration and streaking from 0.65% NaCl solution, as in the following method, but on Patel's selective medium was tried on a number of occasions.

Method of Maas-Geesteranus and Barendsen (137).

This method has been used for the isolation of Agrobacterium gypsophilae from galls on glasshouse roses and carnations in the Netherlands.

The prepared gall pieces were crushed in sterile 0.65% NaCl solution and stood for 24 hours at room temperature before streaking on Nutrient Agar. Isolations were also made after standing for only 12 hours and also for 48 hours.

As mentioned above, streaking from the 0.65% NaCl solution was also carried out on Patel's medium.

Isolation on fleshy root tissues:

Following the method of Ark & Thompson (7) well washed roots of carrot, turnip and parsnip were cut into discs with a sterile scalpel and placed in sterile petri dishes. The freshly cut surfaces were liberally streaked with suspensions of crushed gall prepared as in all the methods outlined above and stood for various periods. The root slices were incubated at room temperature and inspected at 40 x magnification at 7, 14 and 21 days for the appearance of galling symptoms.

Results:

Although the various methods outlined above and combinations of the various methods, were used on

numerous occasions over several seasons, no bacteria were isolated which caused galling on R. multiflora.

Initially, isolated colonies having the general appearance of A. tumefaciens (small, white, circular, glistening, translucent) after 4 to 5 days incubation or the general appearance of A. gypsophilae (small, light yellow, radially lined) after 2 days incubation on Nutrient Agar, were selected and re-streaked on Nutrient Agar plates. In later isolation attempts a greater diversity of regularly appearing colony types were tested.

Testing for the ability to form galls was carried out in a number of ways.

In vitro. Pieces of R. multiflora stem similar to that used for rose stock cutting but containing only 3 nodes and 2 internodes were surface disinfected in 1% sodium hypochlorite solution (surface sterilisation proved impossible with such a large piece of material without affecting its subsequent growth), washed in three changes of sterile water, the ends freshly cut with a sterile scalpel and the bud at the proximal node removed in the manner normal for rose stock cuttings. The proximal end of the stock piece was then inoculated either by dipping in a suspension of the test bacterium in sterile distilled water or in a 24 hour Nutrient Broth culture or inocoulated with bacteria from a plate culture using a platinum loop.

The inoculated stock pieces were placed in sterile tubes wet with sufficient sterile distilled water to

maintain humidity without excessively wetting the base of the stock piece. Uninoculated controls were prepared in a similar manner without inoculation and in some instances controls were prepared with dipping in sterile water or dipping in sterile Nutrient Broth or rubbing with a sterile platinum loop which had been touched onto sterile Nutrient Agar.

In none of these test could any galling be detected in up to 28 days. It was noticed however that apparently normal wound healing callus formation occurred sooner on many of the inoculated stem pieces than on uninoculated and that development of callus of uninoculated stem pieces frequently appeared to coincide with infection of the cut surfaces with contaminating microorganisms, particularly bacteria, probably originating from the epiphytic population on the incompletely sterilised stem piece.

This effect was taken to be due to the presence of growth factor producing organisms such as are frequently recorded in the literature.

Testing in the field was carried out both on established plants and on cuttings.

The cuttings were prepared in the normal manner for making rose stock cuttings. No surface disinfection was carried out but the freshly cut proximal ends were inoculated in the various ways outlined in the <u>in vitro</u> tests above. Five cuttings were inoculated with each test organism and controls were also prepared in batches of five. The cuttings were planted in the open ground in plots of five cuttings for each test.

After 3 to 8 weeks depending on the time of the year these were lifted and examined for signs of galling. No significant difference between the tests and the controls were observed.

Testing on established plants of R. multiflora was also carried out with may of the isolations. Flaps of bark were lifted on the stem of the stocks approximately 3 inches above ground level to expose the torn cambial surface. To avoid contamination this surface was not touched except with the inoculating loop of test bacteria or with the sterile inoculating loop in the case of controls. The bark flaps were lightly replaced and the whole area loosely bound with a polythene strip to exclude further contamination and maintain humidity. The stock tops were either removed at the end of 3 days and the inoculated surface examined in 2 to 3 weeks or the stock tops were left intact until the following spring and then headed back, at the same time as in normal rose production, with examination for galling being carried out 6 to 7 weeks later.

In none of these tests was galling found to be significantly increased due to the presence of the inoculated bacteria.

The cultures under test were maintained until they could be discarded as negative. For short term tests the plates were kept in a refrigerator at slightly above freezing and re-streaked as considered necessary. For tests which took more than a few weeks to evaluate it was felt that repeated sub-culturing may tend to attenuate the virulence of the culture or possibly

select out non-virulent strains. In such cases a sample of the culture to be tested was suspended in sterile double-distilled water in bijou bottles. Such suspensions kept in a refrigerator or at slightly above freezing were found to remain viable for at least two years.

When it appeared unlikely that a single isolation was going to be found that would transmit galling, similar tests were carried out using a suspension in which galls had been crushed and the mixture of organisms arising from the first streaking of such a suspension for inoculating test plants.

Again no increased galling occurred and the test material either developed normal wound callus indistinguishable from that of the controls or the wounded tissue of the test material became brown and dead presumably due to the presence of rot inducing organisms amongst the mixture or organisms present.

Transmission to bud grafted plants:

Some pilot attempts were made to induce proliferation and/or galling on budded plants but as these gave no indication of success and in view of the lack of success in transmitting galling no full trials were carried out.

In total about 20 plants were budded after inoculating the T-opening on the stock with a suspension of crushed gall, a similar number of plants received a small slice of galled tissue in the bottom of the T-opening before the bud was inserted and an equivalent number of controls were budded in the normal manner.

Bud losses were certainly higher in the two treatments compared with the control, but on examination of these failures many showed extensive rotting to be the cause of failure and quite distinct from the bud failure that occurs in association with galling.

Summary:

Despite numerous attempts by a variety of methods no success was achieved in transmitting galling from either below or above ground galls or in isolating a culture of bacteria that would cause galling.

N. Investigation of bacterial isolates from galls inoculated on bud shield explants in vitro.

After the failure as described in appendix M to obtain reasonable sterility of mature stem pieces containing three nodes a further trial was carried out using a single bud, with a bud shield, excised as for bud-grafting. With this smaller stem piece a reasonable percentage (approx. 20%) of explants proved sterile.

The method used was to soak short stem pieces containing 1 node each in 1% sodium hypochlorite for 3 minutes followed by rinsing in three changes of sterile distilled water. The stem pieces were dried in sterile paper tissues and the bud shield excised with a sterile scalpel.

These were placed in test tubes with sterile water-agar slopes without any added nutrients or growth factors to avoid any influence they may have on growth of the explants.

On account of the high percentage of contamination probabably from micro-organisms lodged under the edges of the bud scales and possibly from necrotic tissue on the leaf scar of the subtending leaf each bacterial isolation being tested was inoculated into 20 tubes to include some tubes at least without contamination. Controls were a similar set of 20 tubes without inoculation.

Only a limited number of isolations were tested in this manner as there was no convenient way of

determining which of the inoculated tubes were sterile prior to inoculation and which contained contaminating bacteria.

The isolations tested tended to give one of three different responses.

1. There was no significant difference between the inoculated tubes and the control tubes.

2. Considerably more, and often all, of the inoculated tubes showed blackening and death of the complete bud and shield.

3. Within three weeks an obvious rooting response occurred with approximately twice as many of the inoculated eyes as of the controls. This rooting response occurred in a relatively constant proportion of all the controls of about 25% and in the response No. 1 above occurred in a similar proportion of the inoculated shields.

In the response type No. 3 about 50% of the inoculated shields produced roots and generally the remainder died as a result of contamination.

As no evidence of increased abnormal callus production was observed in any of the inoculated tubes and on account of the high percentage of contamination using bud shield explants, a detailed analysis was not made of these results.

The apparent pattern, which was surprisingly constant was that half the explants failed due to contamination. A quarter were contaminated but remained alive for more than three weeks and generally produced roots and the remaining quarter were in fact

sterile but on inoculation with a suitable bacterial isolation they also produced roots.

The pattern of rooting was not as might have been expected from the proximal end of the shield but the roots emerged from the upper, (external) face of the explant, usually from between the two outer bud scales or occasionally from the pale area above the leaf scar and surrounding the bud itself. Serial sections showed that these roots were generally initiated close to where the bud trace merged with the vascular cylinder of the main stem just above and to the side of the leaf gap. A complete blackening of the bud scales was typical of this response.

Summary:

(1) Difficulties in sterilising whole buds prevented the extensive use of them as test explants.

(2) No evidence of increased abnormal callus production was observed as a result of inoculation with isolates from galls.

(3) Although no detailed analysis was made as it did not appear pertinent to this investigation there was a strong indication that some contaminating bacteria and that some of the inoculated isolations encouraged rooting from the region of the bud.

O. Investigation of effects of Agrobacterium tumefaciens on meristem tips in vitro.

A single experiment comprising 40 inoculated tubes and 40 control tubes was carried out to find the response of meristem tips of lateral buds (Q) to A. tumefaciens in vitro.

The culture used was isolated from galls on peach by Dr. Rainbow, Levin and obtained from him.

The medium was agar slopes of R.M.1964 (150) (Q) without added growth factors.

All the inoculated explants showed some increased water soaked callus growth and some increased in diameter before becoming completely immersed in and killed by excessive bacterial growth.

The controls showed virtually no growth or increase in size.

The differences before becoming immersed in bacteria appeared insufficient to make this a useful test method for isolations.

P. Sectioning and staining methods.

During the course of this study numerous examinations were made of the general anatomy and histology of rose stems and axillary buds, direct from the plant and after culture *in vitro* with various growth factors or after inoculation with various bacterial isolates. Also numerous examinations were made of the early stages of development of bud graft union during the first three weeks after grafting and a more limited number of examinations of mature graft unions after normal or proliferated growth had been made.

The relevant information derived from these examinations is described in the appropriate places in the main text. The examinations of axillary buds and bud shields after culture *in vitro* with various growth factors or after inoculation with various bacterial isolates did not yield any useful information due to the high percentage of contamination with this type of explant (N, Q) which made it difficult to correlate any observed growth changes with their cause.

No anatomical or histological examinations of meristem explants was made.

Methods:

For most of the general anatomical work the bulk of the material was fixed in formalin - acetic acid - alcohol (FAA) and standard procedures (101) followed through an alcohol series to tertiary butyl alcohol and infiltration and embedding in parafin. Woody

tissue was kept to a minimum in preparing the tissue piece. In the case of young bud-grafts the stock wood was trimmed to a thin layer a few cells thick behind the bud-graft union. Bud shields cultured in vitro only had a thin layer of wood attached and examinations of fresh buds were cut in a similar manner. Sectioning was done on a rotary microtome and the ribbons laid out in sequence from left to right and top to bottom along sequentially numbered slides with the numbering at the right hand end. Ribbons were stretched by floating and warming on 4% formalin solution and affixed with Haupt's adhesive.

Parafin was removed with xylene and the sections serially hydrated, stained, dehydrated through alcohol series to xylene and mounted in Canada balsam.

For more detailed histological examination the method of fixing in glutaraldehyde fixative and embedding in glycol methacrylate polymer as described by Feder and O'Brien (62) was used with excellent results. This method is less time consuming and allows thinner sections (1 to 3 microns) with better preservation of cell detail than the usual parafin embedding method. It does not however allow ribbons of serial sections to be cut and is hence not so suitable for general anatomical investigations.

The specimens were fixed, in 3% glutaraldehyde in 0.025M phosphate buffer pH 6.8, at 0^{o}C. Dehydration was through 2-methoxyethanol (2 changes) to 100% ethanol, then to n-propanol and finally to n-butanol, all at 0^{o} C. for 24 hours each.

The monomer mixture found most suitable for embedding for cutting on a rotary microtome was glycol methacrylate 91.5% with 0.5% (w/v) 2,2'-azobis(2-methylproprionitrile) and 8% (v/v) polyethylene glycol. Exclusion of air from the monomer mixture in a gelatin capsule during polymerisation was important and the polymerisation temperature at 40° C had a considerable bearing on the texture of the finished block.

Each section has to be handled individually after sectioning and may be made into temporary or permanent mounts by most usual methods.

As the embedding polymer is water miscible most staining techniques except those using Schiff's reagent can be carried out without having to dissolve the plastic from the tissue section.

For general anatomical examination of older woody graft unions which did not lend themselves to embedding techniques they were merely fixed in FAA and sectioned in a sliding microtome.

Staining was generally done using either toluidine blue or aqueous iodine for rapid general staining. For more detailed examination a basic dye such as safranin counter-stained with an acid dye such as fast green or aniline blue was used.

Q. Tissue, meristem and organ culture in vitro.

Introduction:

All *in vitro* culture work was carried out in 19 x 150 mm rimless Pyrex test tubes with Oxoid caps. Each experiment was carried out using batches of 40 tubes or multiples of 40 held in test tube racks containing 10 rows of 4. Incubation was generally carried out in the dark thus effects due to position of the tubes in the rack were minimised.

Each tube was numbered 1 to 40 on the caps and different coloured caps were used for different racks.

During the initial pilot experiments to arrive at a suitable basal medium and some approximation to a suitable range of growth factors and concentrations of growth factors no attempt at randomisation was made. For convenience, while a large number of growth factors and concentrations were being screened in order to successfully culture the material, concentrations of one factor was varied down the columns to give each of the 4 rows of 10 along the rack a different concentration. A second factor was varied along the rows to give a column of 4 at each concentration or more frequently 5 different concentrations were used to give 2 columns of 4 at each concentration. Thus the interactions of a range of high and low concentrations of two growth factors could be quickly assessed and a general approximation to an optimum area of concentration determined. This optimum area was then able to be assessed in a similar manner against a range of

concentrations of other growth factors until reasonably consistent bud growth of the plant material was achieved.

Methods of preparing explants.

Initially attempts were made to use whole buds taken from the side of mature shoots together with a shield of bark and a thin sliver of wood similar to the bud shields used in the bud-grafting operation. This approach was made in preference to the use of apical meristems as from observations of proliferating buds it had been noted that the normally undeveloped bud primordia in the axils of the third to eighth or ninth bud scales developed and gave rise to a number of the shoots.

Also as it was desired to compare buds from supposedly infected plants with those from presumably uninfected plants the use of actively growing apical meristems would quite possibly produce uninfected explants even from infected plants.

This approach however was soon discontinued as it proved too difficult to adequately surface sterilise a mature lateral bud and adjacent tissues without the sterilising agent killing the bud or seriously affecting its subsequent growth (N).

Sodium hypochlorite, mercuric chloride and formalin solutions were all tried in various concentrations and durations followed by rinsing in three changes of sterile distilled water. The general pattern of response was that those buds which were not surface sterilised and presumably carried a full balanced population of

epiphytic micro-organisms survived, formed callus and grew in vitro for three to four weeks. On the other hand more than 50% of those that were only "partially sterilised" quickly succumbed to rot inducing fungi and bacteria and became blackened and dead within 3 to 4 days. Of the remainder about half showed some contamination and produced callus usually accompanied by a swelling of the bud shield and the other half proved to be sterile with little apparent growth movement. In the case of higher concentrations and longer duration in the sterilising solutions sterile buds were obtained but these showed obvious signs of damage and although they remained at least partly alive for two to three weeks showed no signs of growth.

Although these and other observations indicate a possible role of epiphytic micro-organisms in relation to wound healing this avenue of investigation was not pursued.

The disecting out of sterile apical meristems from the tips of actively growing shoots was chieved with better than 95% success using a sealed sterile cabinet made from perspex. The cabinet consisted of a 2 ft. by 2 ft. perspex box 1 ft. high with all the joints carefully sealed. Access for instruments and materials was through a sliding port in one side which sealed with petroleum jelly when closed.

A recess was built into the centre of the front and the top of the cabinet by cutting a 5" x 6½" rectangle out of both the top and the front. This recess was sealed off from the cabinet with perspex at the back and

sides and a sheet of plate glass at the bottom. This allowed the use of a stereo-microscope with a foot-focusing control to be set up in the recess and focused on the working area inside the cabinet under the plate glass. Access for manipulations and dissecting was through two comfortably placed ports towards the bottom and either side of the front.

A flanged rim on each of these ports enabled one end of a length of 0.0015" thickness polythene layflat tube to be attached with a rubber surgical glove at the other end of each polythene tube. The reason for the very loose flexible link of thin polythene was to equalise any differences in pressure between the inside and outside of the cabinet caused by arm movements. It also allowed much greater freedom of movement and less tiring operation than otherwise would be the case.

Although use of this cabinet was most successful for dissecting out sterile apical meristems and a number of exploratory trials preparatory to determining a suitable medium and concentrations of growth factors was done with it, it was discarded in favour of a third method for the following reasons. As already mentioned apical meristems from actively growing shoots were not really suitable for comparing, the growth factor levels and requirements, between healthy and infected plants because such meristems even from infected plants could well prove to be healthy. Also an actively growing apical meristem could hardly be expected to behave in the same way as a dormant lateral bud starting into growth.

A method was devised to obtain sterile tips of lateral buds without using surface sterilisants. By taking a lateral bud excised with a shield of bark and wood in the same manner as for budding and by peeling back the bark at the same time as slight pressure was applied to the tip of the bud, it was possible to remove all the bud scales and some of the leaf primordia from the bud in one piece. This left the apical dome with 2 to 4 leaf primordia untouched and free of contamination. It was then a simple matter to excise this bud apex with a sterile scalpel and transfer it to a culture tube. For reasons already described an explant with several leaf primordia was preferred although it would have been an easy matter to have excised just the apical dome with the first primordial ridge.

Standardisation of explants.

A presumably infected clone of R. multiflora was used as a source of explants on account of its being available in quantity thus allowing a choice of buds with as closely similar growth potential as possible and all the material would be genetically identical. Of course where comparisons were made with buds from presumably healthy plants these were obtained from seedlings of R. multiflora and due to limitations in material these were not able to be so rigorously selected.

As nothing is known of the factors in roses which determine what type of shoot any one bud will produce if left intact on the plant or whether these factors

already exist in the dormant bud similar buds were chosen in the following manner.

A block of plants, of the R. multiflora clone AND, grown from a single source were cut back to 15 inches from the ground in January. These plants produced shoots from the eyes immediately below where they had been cut which were closely similar in age, thickness, length and general appearance. At the end of the following April 600 of these shoots were selected as closely similar as possible. At the base of each shoot is a difficult to determine number of buds and bud primordia which have been formed in the axils of the parent bud cataphylls. In order to avoid these the nodes on each shoot were counted from a point 1" above the base of the shoot. A section was cut out of each shoot from just above the 4th node to just below the 15th node. These pieces were then examined closely and selected visually for conformity. Those selected were then tested for length and those varying from the median by more than ± 10% were discarded. Those left were weighed collectively to determine a mean and then weighed individually, discarding those that varied from the mean by more than ± 10%. The less than 200 pieces remaining were then coolstored and drawn on as required.

As the experiments were done during April to October in three successive years a similar method of selection of material was used each year.

Basal Mediums.

Initially White's Tissue Culture medium (197, 198)

(Difco 5784) was used but development on this appeared slow and Dr. R.F. Elliott, who was working on a similar project at the time, suggested Murashige & Skoog's basal medium. The revised version of this medium (RM 1964) as published by Linsmaier & Skoog (135) comprising mineral salts (major and minor elements) together with sucrose 30 g/l, thiamine HCL 0.400 mg/l, myoinositol 100 mg/l and agar 10 g/l was adopted as the basal medium with improved growth rates. This medium differs from others in general use in that it is much higher in mineral salt content, particularly nitrogenous and potassium salts.

Double-distilled water was used for all mediums for culturing explants.

A number of earlier experiments were done using both White's Tissue Culture medium and R.M. 1964 without agar as a liquid solution and the explants supported on a strip of filter paper bent to form a platform above the solution but with the ends of the strip dipping into it.

It was found however, particularly with the eye shield explants, that contaminating bacteria presumably from the epiphytic population were not apparent on the filter paper but appeared to be causing some growth modifications in the explants. In order to quickly ascertain the presence of such contaminants and exclude affected explants from the results an agar base was used for all the experiments described. Ten mls of agar medium was used per tube and this was sloped in the tube in order to drain any excess

condensation liquid away from the explant.

Supplementary growth factors.

Satisfactory levels of auxin and cytokinin were first investigated using 3-indoleacetic acid (IAA) for the auxin component and benzyl adenine (BA) (6-benzylaminopurine) for the cytokinin component.

A series of experiments were carried out using a series of dilutions of BA between $2X10^{-5}M$ and $2X10^{-6}M$ coupled with a dilution series of IAA between $10^{-4}M$ and $10^{-5}M$. The highest concentration of BA was also used with no added IAA and similarly the highest concentration of IAA was used without BA.

This range of concentrations was chosen to vary about the optimal concentrations for the production of shoots on tobacco callus as reported by Linsmaier & Skoog (135). The concentrations of BA and IAA were paired so that as the BA increased the IAA decreased. Three replicates were used of 10 different combinations of IAA and BA concentrations with 4 tubes of each combination in each replicate. The explants consisting of approximately 1 mm tips of lateral buds with 2 to 4 leaf primordia were inserted on 27/5/69 and incubated at 21° to 24° C. The condition and appearance of each explant was recorded at time of insertion and each week thereafter until 1/7/69 noting changes in appearance, development and type of callus and movement of leaf primordia.

Despite careful standardisation of material used for explants there was considerable variation in performance between explants in any one treatment

although general trends over the range of treatments were apparent.

In the early stages greatest development of leaf primordia together with callus development of small tightly packed cells of watersoaked appearance occurred at the higher concentrations of BA with low IAA. Also $2X10^{-5}M$ BA without IAA gave similar development.

On the hand the relatively high IAA concentrations with lower BA produced little or no development of the leaf primordia with some white callus of large loosely packed cells. With IAA at $10^{-4}M$ and no added BA there was little callus development apart from an unusual growth of long root-hair-like cells with lengths up to 20 times their diameter. These were formed by the elongation of small scattered patches of callus cells on the surface of the explant.

By the time of the last observation some of the cultures were becoming senescent. By this time the general pattern of growth was obvious. The extent of callus growth varied from approximately 3 mm diameter with little movement of leaf primordia and apical meristem at the end of the series with higher IAA and lower BA to, at the other end of the series where there was high BA and lower IAA, massive 7 to 8 mm diameter callus growth on which the initial development of leaf primordia had been suppressed, overgrown or displaced by the disorganised callus growth. The treatment with high BA ($2X10^{-5}M$) and no IAA showed less disorganised callus growth but the explants had generally increased in size to 4 to 5 mm diameter but

this had occurred in part by growth of the whole explant rather than callus formation on the surface. The leaf primordia were still alive and green having increased in size to 1 to 1½ mm in length but made no progress beyond that. On some of these explants up to 18 new leaf primordia had been produced around the apical meristem.

It was therefore apparent that BA was necessary for growth of the leaf primordia anddevelopment of the apical meristem and IAA tended to increase callus formation at least in the presence of BA and suppress organised growth of the explant.

The treatment with $2X10^{-5}M$ BA appeared to be causing damage to the tissues where they were in contact with the medium. On account of the relatively high mineral salt concentrations in RM 1964 a small experiment was carried out using a dilution series of the basal medium with the same strength of BA ($2X10^{-5}M$). The dilution of the basal medium appeared to make little difference to the damage to the tissues.

In a similar manner to the BA plus IAA experiment a series of experiments were carried out using a lower dilution range of BA from $2X10^{-6}M$ to $2X10^{-8}M$.

Each experiment tested this range of BA concentrations against a different growth factor used by other workers in tissue and organ culture.

The growth factors tested were guanosine, cytidine, adenine, nicotinic acid, naphthaleneacetic acid, calcium pantothenate and pyridoxine-HCL. These were all growth factors used by Tulecke, Taggart and Colavito (192)

for cultures of rose tissue and these were tested at the same concentrations used by them.

At these concentrations no improvement in meristem growth was observed over that achieved with BA alone. Various types of callus development with the different treatments were noted including the formation of greenish callus nodules and green thalloid tissue growth but nothing which appeared to add to the aim of establishing meristems in vitro for testing for multiple shoot production.

The effect of gibberellic acid.

The same range of BA concentration $2X10^{-6}M$ to $2X10^{-8}M$ were then tested with gibberellic acid (GA_3) at concentrations from $10^{-5}M$ to $10^{-6}M$. Two weeks after inserting the explants the leaf primordia in 8 of the 10 tubes in the treatment containing $2X10^{-7}M$ BA plus $10^{-5}M$ GA_3 had elongated considerably to between 5 and 13 mm long. The original explants had consisted of approximately 1 mm tips of lateral buds.

The leaves formed by the elongating primordia were very narrow and attenuated with up to 5 equally attenuated leaflets. The experiment was repeated with similar results.

The appearance of the attenuated leaves and the basal callus was similar to some effects of hyperauxiny. The source of explants was from a clone of R. multiflora known to give rose wilt virus (sensu Fry & Hammett) and proliferation symptoms when budded and that both these conditions produced some symptoms similar to those caused by hyperauxiny.

As no auxin had been added to the medium it was presumed that any excess auxin present must have been formed by the explant. It was decided to carry out an experiment using the same conditions but with added antiauxin. As it was desirable that the anti-auxin itself should have a minimum physiological effect on the explant, p-chlorophenoxyisobutyric acid was chosen, as it had no known physiological effect, other than its competitive inhibition at the active sites and its failure to act as an auxin due to steric hindrance of its isobutyric side chain.

The experiment was set up using five concentrations of p-chlorophenoxyisobutyric acid 10^{-4}M, 10^{-5}M, 10^{-6}M, 10^{-7}M and 10^{-8}M all with $2X10^{-7}$M BA and $1C^{-5}$M GA_3.

At the end of two weeks all the explants in 10^{-4}M p-chlorophenoxyisobutyric acid had failed, all those in 10^{-5}M and 10^{-6}M showed growth of the leaf primordia to 3 to 6 mm long with 3 to 5 leaflets spreading and of approximately normal R. multiflora shape. Those in the weaker concentrations had either made little movement or produced narrow attenuated leaves similar to those without anti-auxin (75).

Subsequent to these findings a paper was published by Dr. R.F. Elliott (60) showing that the presence of GA_3 in the medium caused R. multiflora apices to grow rapidly and form abnormal attenuated leaves. Her work was done using dissected out terminal meristems of actively growing shoots of seed-raised plants in sterilised medium so there could be no possibility of the effect being caused by systemic infection with

viruses or proliferation.

To test the lateral bud meristems of the presumably infected stock used in the previous experiments a further experiment was set up using the same type of explants as previously with $2X10^{-7}$M BA but no GA_3. The explants produced normal R. multiflora shaped leaves.

The performance of explants from lateral buds taken from mature stem pieces of seed-raised R. multiflora was then tested using $2X10^{-7}$M BA with and without 10^{-5}M GA and with 10^{-5}M GA plus 10^{-6}M p-chlorophenoxy-isobutyric acid.

No difference could be observed between the performance of these presumably uninfected explants and the performance of those from the presumably infected clone of commercial stock.

Summary:

(1) It was found that 1 mm tips with 2 to 4 leaf primordia taken from lateral buds of R. multiflora could be induced to form leaves on an agar medium containing mineral salts and essential organic constituents as defined by Linsmaier & Skoog in RM 1964 together with $2X10^{-7}$M BA.

(2) No difference could be found in growth requirements for explants from lateral buds of presumably infected stock to the growth requirements of explants from lateral buds of seedling grown stock presumably uninfected.

(3) The presence of 10^{-5}M GA_3 in the medium caused attenuated leaf growth similar to some effects caused

by hyperauxiny but this effect could be overcome by the inclusion of 10^{-5}M and 10^{-6}M p-chlorophenoxyisobutyric acid in the medium.

(4) The further work reported here, showing that the narrow attenuated leaf growth under these conditions is caused by the presence of GA_3 in the medium, is contrary to the inference in earlier reported work (75) that this effect was due to hyperauxiny occurring in infected explants.

BIBLIOGRAPHY
INCLUDING NOTES

1. AARON-DA CUNHA, M.I. 1969. Sur la libération par les Rayon-X, d'un principe tumorigène contenu dans les tissus de crown gall de Tabac. C.R. Acad. Sci. Paris. 268 D : 319.

2. AHUJA, M.R. 1965. Genetic control of tumor formation in higher plants. Q. Rev. Biol. 40 (4) : 329-340.

3. AKRIGG, A., AYAD, S. & BLAIRE, J. 1969. Uptake of DNA by competent bacteria - A possible mechanism. J. theoret. Biol. 24 : 266-272.

4. ALLINGTON, W.B., STAPLES, R. & VIEHMEYER, G. 1968. Transmission of rose rosette virus by the eriophyid mite Phyllocoptes fructiphilus. J. econ. Ent. 61 : 1137-40.

5. AMES, I.M., RICE, T.B. & SMITH, K.M. 1969. Inhibition of tumor induction by auxin in totally debudded Nicotiana glauca X langsdorffii. Pl. Physiol. 44 : 305-307.

6. ANONYMOUS. 1962. Rep. Dep. Agric. N.S.W. 1960-61.

7. ARK & THOMPSON. 1961. Detection of hairy root pathogen, Agrobacterium rhizogenes by the use of fleshy roots. Phytopathology 51 : 69-71.

8. ASEN, S. & HAMNER, C.L. 1953. Effect of growth-regulating compounds on development of basal shoots of greenhouse roses. Bot. Gaz. 115 : 86-89.

9. BAILEY, N.T.J. 1959. Statistical methods in biology. The English University Press Ltd. London.

10. BAKER, K.F. & SNYDER, W.C. Eds. 1965. Ecology of soil borne plant pathogens. John Murray, London. (Int. Symp. Berk. Calif. 1963).

11. BASIT, A.A., FRANCKI, R.I.B. & KERR. A. 1970. The simultaneous transmission of a plant pathogenic bacteria and a virus from rose by grafting and mechanical inoculation. Aust. J. biol. Sci. 23 (2) : 493-496.

12. BATCHELOR, L.D. Cal.-Poly. Calif. (personal communication).

13. BAUMAN, G. 1965. The transmission of the proliferation virus to apple seedlings in the glasshouse. Prelim. rept., NachrBl. dtsch. PflSchDienst. Braunschweig. 17 : 73-75. Hort. Abst. 35 : 5400.

14. BAYER, M.H. & AHUJA, M.R. 1968. Tumor formation in Nicotiana: Auxin levels and auxin inhibitors in normal and tumor-prone genotypes. Planta. 79 : 292-298.

15. BEARDSLEY, R.E. 1955. Phage production by crown gall bacteria and the formation of plant tumors. Am. Nat. 89 : 175-176.

16. BEARDSLEY, R.E. 1960. "Lysogenicity in Agrobacterium tumefaciens". J. Bact. 80 : 180-187.

17. BENDER, E. & BRUCKER, W. 1956. Studien zur zellfreien Tumorübertragung an Pflanzen I. Z. Bot. 44 : 531-542.

18. BIEBER, J. & SARFERT, E. 1968. Zur Frage der Tumorbildung durch Desoxyribonukleinsäure aus Agrobacterium tumefaciens. Phytopath. Z. 62 : 323-326.

19. BLACK, L.M. 1964. Wound tumors of plants. Proc. Conf. Abnorm. Growth Pl., Univ. Calif. pp. 5-9.

20. BLACK, L.M. 1965. Physiology of virus induced tumors in plants. Handb. PflPhysiol. 15 (2) : 236 - 266.

21. BLOCH, R. 1941. Wound healing in higher plants. Bot. Rev. 7 : 110-146.

22. BLOCH, R. 1952. Wound healing in higher plants II. Bot. Rev. 18 : 655-679.

23. BLOCH, R. 1953. "Abnormal plant Growth". Brookhaven Symp. Biol. No. 6. Abnormal & pathological plant growth.

24. BLOCH, R. 1965. Abnormal development in plants : A survey. Handb. PflPhysiol. 15 (2) : 156-183.

25. BOELEMA, B.H. 1969. Resistance of rose rootstocks to crown gall (Agrobacterium tumefaciens). Neth. J. Pl. Path. 74 : 147-150

26. BOS, L. & GRANCINI, P. 1965. Some experiments and considerations on the identification of witches broom viruses especially in clovers in The Netherlands and in Italy. Neth. J. Pl. Path. 71 Suppl. 1 : 1-20.

27. BOYD, R.J., HILDEBRANDT, A.C. & ALLEN, O.N. 1971. Retardation of crown gall enlargement after bacteriophage treatment. Pl. Dis. Reptr. 55 (2) : 145-148.

28. BRADFORD, F.C. & SITTON, G.B. 1929. Defective graft unions in the apple and pear. Mich. St. Agric. Stn. Tech. Bull. 99 : 1-106.

29. BRAUN, A.C. 1941. Development of secondary tumors and tumor strands in the crown gall of sunflowers. Phytopathology 31 : 135-149.

30. BRAUN, A.C. 1943. Studies on tumor inception in the crown gall disease. Am. J. Bot. 30 : 674-677.

31. BRAUN, A.C. 1947. Thermal studies on the factors responsible for tumor initiation in crown gall. Am. J. Bot. 34 : 234-240.

32. BRAUN, A.C. 1948. Studies on the origin and development of plant teratomas incited by the crown gall bacterium. Am. J. Bot. 35 : 511-519.

33. BRAUN, A.C. 1953. Studies on the origin of the crown gall tumor cell. Brookhaven Symp. Biol. No. 6, Abnormal & pathological plant growth pp. 115-127.

33a. BRAUN, A.C. 1953. Bacterial and host factors concerned in tumor morphology in crown gall. Bot. Gaz. 114 : 363.

34. BRAUN, 1959. A demonstration of the recovery of the crown gall tumor cell with the use of complex tumors of single-cell origin. Proc. natn. Acad. Sci. U.S.A. 45 : 932-938.

34a. BRAUN, 1962. Tumor inception and development in the crown gall disease. A. Rev. Pl. Physiol. 13 : 533-558.

35. BRAUN & WHITE. 1943. Bacteriological sterility of tissues derived from secondary crown gall tumors. Phytopathology. 33 : 85-100.

35a. BRIEN, R.M. & DINGLEY, J.M. 1951. A revised list of plant diseases recorded in New Zealand. Bull. N.Z. Dep. scient. ind. Res. 101 : 62pp.

36. BROWN, N.A. & GARDNER, F.E. 1936. Galls produced by plant hormones including a hormone extracted from Bacterium tumefaciens. Phytopathology 26 : 708-713.

37. BROWN, C.L. & SAX, K. 1962. The influence of pressure on the differentiation of secondary tissues. Am. J. Bot. 49 (7) : 683-691.

38. BRUNDRETT, S. 1929. Dieback in roses. Aust. Rose A. p. 155.

38a. BRUNNER, L. 1970. Preformed phage released by ultra-violet irradiation of lysogenic Agrobacterium tumefaciens. Diss. Abstr. B. 31 (2) : 819. PhD. Thesis Penn. St. Univ.

39. BUCK, G.J. 1953. The histological development of the bud graft union in roses. Proc. Am. Soc. hort. Sci. 62 : 497-502.

40. BUCK, G.J. & HEPPEL, B.J. 1970. Bud graft incompatability in Rosa. Rroc. Am. Soc. hort. Sci. 95 : 442-446.

40a. CAMERON, H.R. 1970. Pseudomonas content of cherry trees. Phytopathology. 60 : 1343-1346.

41. CAMMACK, R.H. 1966. Soil-borne viruses in rose. Pl. Path. 15 : 47-48.

42. CARLSON, M.C. 1933. Comparative anatomical studies of 'Dorothy Perkins' and 'American Pillar' roses I Anatomy of canes II Origin and development of adventitious roots in cuttings. Contr. Boyce Thompson Inst. Pl. Res. 5 : 313-330.

43. CATARINO, F.M. & RESENDE, F. 1966. Kinetin-like substances produced by plant tumors. Port. Acta. biol. 9 : 373-382.

44. CLARKE, G., DYE, M.H. & WAIN, R.L. 1959. Occurrence of 3-indolylacetic acid and 3-indolylcarboxylic acids in tomato crown gall tissue extracts. Nature Lond. 184 : 825-826.

45. CLOWES, F.A.L. 1961. Apical meristems. Oxf. Bot. Monogr. (2).

46. COLHOUN, J. (unpublished report) Manchr. Univ.

47. COPES, D. 1969. Graft union formation in Douglas Fir. Am. J. Bot. 56 : 285-289.

47a. DEEP, L.W. & YOUNG, R.A. 1965. The role of preplanting treatments with chemicals in increasing the incidence of crown gall. Ore. St. Univ. Corwallis. Phytopathology (Abst.) 55 : 212-216. Hort. Abst. 35 : 5431.

48. de ROPP, R.S. 1947. The isolation and behaviour of bacteria-free crown gall from primary galls of Helianthus annuus. Phytopathology 37 : 201-206.

49. de ROPP, R.S. 1947. The growth promoting and tumefacient factors of bacteria-free crown gall tumor tissue. Am. J. Bot. 34 : 248-261.

50. de ROPP, R.S. 1948. The growth-promoting action of bacteria-free crown gall tumor tissue. Bull. Torrey bot. Club. 75 : 45-50.

51. de ROPP, R.S. 1948. The movement of crown gall bacteria in isolated stem fragments of sunflower. Phytopathology 38 : 993-998.

52. DICKEY, R.S. 1961. Relation of some edaphic factors to Agrobacterium tumefaciens. Phytopathology. 51 : 607-614.

53. DICKEY, R.S. 1962. Efficacy of five fumigants for the control of Agrobacterium tumefaciens at various depths in the soil. Pl. Dis. Reptr. 46 (2) : 73-76.

54. DIMOCK, A.W. 1951. Bud transmission of Verticillium in roses. Phytopathology 41 : 781-784.

54a. DINGLEY, J.M. 1969. Records of plant diseases in New Zealand. Bull. N.Z. Dep. scient. ind. Res. 192 : 298 pp.

55. DYE, D.W. (unpublished communication) Plant Diseases Division, Auckland.

56. DYE, M.H. 1959. Studies on plant growth responses induced by Agrobacterium tumefaciens. PhD Thesis Univ. Lond.

57. DYE, M.H., CLARKE & WAIN. 1962. Investigations on the auxins in tomato crown gall tissue. Proc. R. Soc. (Ser. B. Biol. Sci.) 155 : 478-492.

58. EL KHALIFA, M.D. & LIPPINCOTT, J.A. 1968. The influence of plant-growth factors on the initiation and growth of crown gall tumors on primary pinto bean leaves. J. exp. Bot. 19 (61) : 749.

58a. EL KHALIFA, M.D. & LIPPINCOTT, J.A. 1968. Quantitative measurements of interactions between crown gall tumors and the pinto bean host. Am. J. Bot. 55 : 382-91.

59. ELLIOT, R.G. The Australasian Rose Book. p.178.

60. ELLIOTT, R.F. 1970. Axenic culture of meristem tips of Rosa multiflora. Planta. 95 : 183-186.

61. ESAU, K. 1953. Plant anatomy. John Wiley & Sons.

62. FEDER, N & O'BRIEN, T.P. 1968. Plant microtechnique : some principles and new methods. Am. J. Bot. 55 (1) : 123-142.

63. FENWICK, E.L. & SHEAT, D.E.G. 1968. Growth responses of sunflower hypocotyl disks inoculated with Escherichia coli and Agrobacterium tumefaciens. I morphological observations. II Analyses of increases in weight. Pl. Cell Physiol. 9 : 285-305.

64. FLETCHER, W.E. 1964. Peach bud graft unions in Prunus besseyi. Proc. Pl. Prop. Soc. 14 : 265-272.

65. FOSTER, D.M. & WEBER, D.J. 1969. Free amino acid pools and enzymes in teratoma and habituated tobacco tissue. Physiologia Pl. 22 (6) : 1263-1272.

66. FRAZIER, N.W. 1963. Strawberry as a possibly useful indicator host of rose viruses. Pl. Dis. Reptr. 47 (7) : 585-586.

67. FROST, R.R. 1971. Importance of rose virus diseases. Gdnrs. Chron. 169 (12) : 6-7.

68. FRY, P.R. & HAMMETT, K.R.W. 1971. Rose wilt virus in New Zealand. N.Z. Jl. agric. Res. 14 (3) : 735-743.

69. FUJINAGA, K. & GREEN, M. 1966. Proc. natn. Acad. Sci., U.S.A. 57 : 806-813.

70. FULFORD, R.M. 1965. The morphogenesis of apple buds. I: the activity of the apical meristem. Ann. Bot. N.S. 29 : 167-180.

71. FULFORD, R.M. 1966. The morphogenesis of apple buds. II: The development of the bud. Ann. Bot. N.S. 30 : 25-38.

72. GALSKY, A.G. 1969. Studies on gibberellin production by Agrobacterium sp. and on the generalised mechanism of gibberellin action on barley endosperm. Diss. Abstr. B.4524. Ph.D. Thesis. Northwestern Univ.

73. GALSKY, A.G. & LIPPINCOTT, J.A. 1967. Production of a gibberellin-like substance by strains of Agrobacterium tumefaciens. Pl. Physiol. Lancaster. 42 : S-29.

74. GARDNER, P.C. June 1966, Nov. 1967. (unpublished reports).

75. GARDNER, P.C. 1970. What's wrong with roses? - an hypothesis. N.Z. Rose A. pp 122-128.

76. GARRISON, R. 1949. Origin and development of axillary buds: Syringa vulgaris. Am. J. Bot. 36 : 205-213.

77. GARRISON, R. 1949. Origin and development of axillary buds : Betula papyrifera Euptelea polyandra. Am. J. Bot. 36 : 379.

78. GARRISON, R. 1955. Studies in the development of axillary buds. Am. J. Bot. 42 : 257.

79. GEE, M.M., SUN, C.N. & DWYER, J.D. 1967. An electron microscope study of sunflower crown gall tumor. Protoplasma. 64 : 195-200.

80. GRIEVE, B.J. 1931. 'Rose wilt' and 'Dieback'. A virus disease of roses occurring in Australia. Aust. J. exp. Biol. med. Sci. 8 : 107-121.

81. GRIEVE, B.J. 1942. Further Observations on Rose Wilt Virus. Proc. R. Soc. Vict. N.S. 54 (2) : 229-238.

82. GRIFFIN, G.D., ANDERSON, J.L. & JORGENSON, E.C. 1968. Interaction of Meloidogyne hapla and Agrobacterium tumefaciens in relation to raspberry cultivars. Pl. Dis. Reptr. 52 : 492-493.

83. GUILLÉ, E. & QUETIER, F. 1970. Le "Crown Gall" : modèle expérimental pour l'étude du mécanisme de la transformation tumorale. C.R. Acad. Sci. Paris. 270 D : 3307-3310.

84. HAMMETT, K.R.W. 1971. Symptom differences between rose wilt virus and verticillium wilt of roses. Pl. Dis. Reptr. 55 (10).

85. HARRIS, D. 1971. Importance of rose virus diseases. Gdnrs' Chron. 169 (12) : 6-7.

86. HARRISON, B.D. 1967. Transmission of strawberry latent ringspot virus by Xiphinema diversicaudatum. (Nematoda). Ann. appl. Biol. 60 : 405-409.

87. HEATH, O.V.S. 1970. Investigation by experiment. Edward Arnold (Publishers) Ltd.

88. HEBERLEIN, G.T. & LIPPINCOTT, J.A. 1965. Photo reversible Ultraviolet enhancement of infectivity in Agrobacterium tumefaciens. J. Bact. 89 : 1511-1514.

89. HEBERLEIN, G.T. & LIPPINCOTT, J.A. 1967. Ultraviolet induced changes in the infectivity of Agrobacterium tumefaciens. J. Bact. 93 : 1246.

90. HEBERLEIN, G.T. & LIPPINCOTT, J.A. 1967. Enhancement of Agrobacterium tumefaciens infectivity by mitomycin C. J. Bact. 94 : 1470.

91. HELGESON, J.P. & LEONARD, N.J. 1966. Cytokinins: Identification of compounds isolated from Corynebacterium fascians. Proc. natn. Acad. Sci. U.S.A. 56 : 60-63.

92. HESS, D. 1969. Versuche zur Transformation an höheren Pflanzen: Induktion und Konstarte. Weitergabe der Anthocyansynthese bei. Petunia hybrida. Z. PflPhysiol. 60 (4) : 348-358.

93. HESS, D. 1970. Transformation experiments in higher plants; Genetic characterisation of some probably transformed plants. Z. PflPhysiol. 63 (1) : 31 et seq.

94. HESS, D. 1970. Molekulare Genetik bei höheren Pflanzen. Ber. dt. bot. Ges. 83 : 279-300.

95. HESS, D. 1970. Transformation in higher plants. Z. PflPhysiol. 63 (5) : 461-467.

95a. HODGSON, R., RIKER, A.J. & PETERSON, W.H. 1947. A wilt inducing toxic substance from crown gall bacteria. Phytopathology. 37 : 301-318.

96. HORTICULTURAL RESEARCH CENTRE - LEVIN. 1967. Minutes of meeting to discuss rose production problems.

97. HOURSIANGOU-NEUBRUN, D. & PUISEUX-DAO, S. 1969. Premières observations mettant en évidence la possibilité pour des phages de l' Agrobacterium tumefaciens d' être responsables du crown gall. C.R. Acad. Sci. Paris. 268 D (11) : 1493.

98. HUONG, C.C. 1967. Chromosoma. 23 : 162-172.

99. HUTTON, B. 1970. Stunt disease - and work at Shardlow. Gdnrs' Chron. 168 (1) : 31-33.

100. JACOBS, G., BORNMAN, C.H. & ALLAN, P. 1968. Tissue culture studies on rose. Use of pith explants. S. Afr. J. Agric. Sci. 11 : 673-678. Hort. Abst. 40 : 1754.

101. JENSEN, W.A. 1962. Botanical Histochemistry. W.H. Freeman and Co. San Francisco.

102. JOHANSEN, D.E. 1940. Plant microtechnique. McGraw-Hill Book Co. Inc. New York.

103. KATZNELSON. 1965. Nature and importance of the Rhizosphere. Ecology of Soil Borne Plant Pathogens. Eds., Baker & Snyder. John Murray, London.

104. KATZNELSON, H. & COLE, S. 1965. Production of Gibberellin-like substances by bacteria and Actinomycetes. Can. J. Microbiol. 11 : 733-741.

105. KATZNELSON & SIROS. 1961. Auxin production by species of Arthrobacter. Nature, Lond. 191 : 1323-1324.

106. KATZNELSON, SIROS & COLE. 1962. Production of a gibberellin-like substance by Arthrobacter globiformis. Nature, Lond. 196 : 1012-1013.

107. KEANE, P.J., KERR, A. & NEU, P.B. 1970. Crown gall of stone fruit II. Identification and nomenclature of Agrobacterium isolates. Aust. J. biol. Sci. 23 (3) : 585-596.

108. KEENER, P.D. 1945. Mycoflora of buds. Science. N.Y. 102 (2650) : 383.

109. KERR, A.N. 1965. The Growth and development of spontaneous plant tumors. (Hand. Pfl.Physiol. 15 (2) : 184-208.

110. KERR, A.N. & SMITH, H.H. 1953. Genetic tumors in Nicotiana hybrids. Brookhaven Symp. Biol. No. 6 Abnormal & pathological plant growth pp. 53-78.

111. KERR, A. 1969. Crown Gall of Stone Fruit I: Isolation of Agrobacterium tumefaciens and related species. Aust. J. biol. Sci. 22 : 111-116.

112. KERR, A. 1969. Transfer of virulence between isolates of Agrobacterium. Nature, Lond. 223 : 1175-1176.

113. KERN, H. 1965. Investigations on the genetic transformation between Agrobacterium tumefaciens and Rhizobium species. I Transfer of the ability to induce plant tumors to Rhizobium species. Arch. Mikrobiol. 51 (2) : 140.

114. KERN, H. 1965. Investigations on the genetic transformation between Agrobacterium tumefaciens and Rhizobium species. II Comparative morphological, physiological and biochemical studies of the partners in a transformation. III Compartive analyses of DNA. Arch. Mikrobiol. 52 (3) 206.

115. KERN, H. 1965. Untersuchungen zur genetischen Transformation zwischen Agrobacterium tumefaciens und Rhizobium. Arch. Mikrobiol. 52 (4) : 325.

116. KLAMBT, D.G., THIES, G. & SKOOG, F. 1966. Isolation of cytokinins from Corynebacterium fasciens. Proc. natn. Acad. Sci. U.S.A. 56 : 52-59.

117. KLEIN, R.M. 1953. Mechanisms of crown gall induction. Brookhaven Symp. Biol. No. 6. Abnormal & pathological plant growth pp 97-114.

118. KLEIN, R.M. 1965. The physiology of bacterial tumors in plants and of habituation. Hand. PflPhysiol. 15 (2) : 209-235.

119. KLEIN, D.T. & KLEIN, R.M. 1953. Transmittance of tumor-inducing ability to avirulent crown gall and related bacteria. J. Bact. 66 : 220-228.

120. KLEIN, D.T. & KLEIN, R.M. 1956. Quantitative aspects of transformation of virulence in Agrobacterium tumefaciens. J. Bact. 72 : 308.

120a. KLEIN, R.M. & LINK, G.K.K. 1955. The etiology of crown gall. Q. Rev. Biol. 30 : 207.

121. KLESSER, P.J. (personal comments) Hort. Res. Inst. Pretoria.

122. KLINKOWSKI, M. Die Rosenwelke. Pflanzliche virologie. 2 (2) : 278-279.

123. KORANT, B.D. 1969. Characterisation of the deoxyribonucleic acids of Agrobacterium tumefaciens, its phage and Helianthus crown gall tumors. Diss. Abst. B. 30 (11) 5161-B Ph.D. Thesis Penn. St. Univ.

124. KOSUGE, T. & WILSON, E.E. 1965. The conversion of L-tryptophan to indole-3-acetic acid by a phytopathogenic bacterium Pseudomonas savastonoi. Pl. Physiol. 40 Suppl. 59.

125. KOVOOR, A. 1967. Sur la transformation de tissus normaux de Scorsonère provoquée in vitro par l'acide desoxyribonucleique d' Agrobacterium tumefaciens. C.R. Acad. Sci. Paris. 265 D : 1623-1626.

126. KUPILA-AHVENNIEMI, S. & THERMAN, E. 1971. First DNA synthesis around sterile and crown gall inoculated wounds in Vicia faba. Physiologia Plant. 24 : 23-26.

127. KURKDJIAN, A. 1968. Apparition de phages au cours de l'induction des tumeurs du crown gall. J. Microsc. 7 : 1039-1044.

128. KURKDJIAN, A., BEARDSLEY, R. & MANIGAULT, P. 1968. Bacteriophages d' Agrobacterium tumefaciens II Souche B 6 m. Lysogene dans les tissus de Pisum sativum et effet de la glycine sur la production des phages. Annls. Inst. Pasteur. Paris. 114 : 555-561.

129. LEFF, J. & BEARDSLEY, R.E. 1969. The induction of crown gall tumors by viral DNA. Abstr. XI Int. Bot. Congr. 125.

130. LEVINE, M. 1923. Studies on plant cancers. V. Leafy crown galls on tobacco plants resulting from Bacterium tumefaciens inoculations. Phytopathology 13 : 107-116.

131. LEVINE, M. 1937. Tumors of tobacco hybrids. Am. J. Bot. 24 : 250-256.

132. LIBBERT, E., KAISER, W. & KUNERT, R. 1969. Interactions between plants and epiphytic bacteria regarding their auxin metabolism VI The influence of the epiphytic bacteria on the content of extractable auxin in the plant. Physiologia Pl. 22(2) : 432-439.

133. LIBBERT, E. & RISCH, H. 1969. Interactions between plants and epiphytic bacteria regarding their auxin metabolism. V. Isolation and identification of the IAA-producing and IAA-destroying bacteria from pea plants. Physiologia Pl. 22 : 51-58.

134. LIBBERT, E., WICHNER, S., SCHIEWER, V., RISCH, H. & KAISER, W. 1966. The influence of epiphytic bacteria on auxin metabolism. Planta 68 : 327-334.

135. LINSMAIER, E.M. & SKOOG, F. 1965. Organic growth factor requirements of tobacco tissue cultures. Physiologia Pl. 18 : 100-127.

136. LIPPINCOTT, B.B. & LIPPINCOTT, J.A. 1969. Bacterial attachment to a specific wound site as an essential stage in tumor initiation by Agrobacterium tumefaciens. J. Bact. 97 : 620-628.

136a. LOCKE, S.B., RIKER, A.J. & DUGGAR, B.M. 1938. Growth substances and the development of crown gall. J. agric. Res. 57 : 21-39.

137. MAAS-GEESTERANUS, H.P. & BARENDSEN, H. 1966. Hostplants of Agrobacterium gypsophilae. Neth. J. Pl. Path. 72 : 231-232.

138. MALENIN, I. Some histological and morphological changes in Vitis vinifera induced by Agrobacterium tumefaciens (Smith and Towns) Conn. Grad. lozar. Nauka. 6 (6) : 69-76. Hort. Abstr. 40 (3).: 5960.

139. MARCUSSEN, K.H. 1971. Rose Production problems. Nursery Stock Research and Extension Advisory Committee report. N.Z. Dept. of Agric. Wellington.

140. MASSEY, L.M. 1950. Crown gall on roses. Am. Rose A. 35 : 145-153.

141. MILNE, K. (Unpublished communication) Massey Univ., Palmerston North.

142. MILO, G.E. & SRIVASTAVA, B.I.S., 1969. RNA-DNA hybridisation studies with crown gall bacteria and tobacco tumor tissue. Biochem, biophys. Res. Commun. 34 : 196-199.

143. MOREY, D. (Unpublished communication) General Bionomics, California.

144. MOSSE, B., & LABERN, M.V. 1960. The structure and development of vascular nodules in apple bud unions. Ann. Bot. NS 24 : 500-507.

145. MUNCIE, J.H. 1926. A study of crown gall caused by Pseudomonas tumefaciens on rosaceous hosts. Iowa St. Coll. J. Sci. 1 : 67-117.

146. MUNCIE, J.H. & PATEL, M.K. 1929. Potency and specificity of a lytic principal (bacteriophage) obtained from Pseudomonas tumefaciens. Phytopathology 19 : 98.

147. MUNCIE, J.H. & SUIT, R.F. 1930. Studies of crown gall overgrowths and hairy-root on apple nursery stock. Iowa St. Coll. J. Sci. 4 : 263-313.

148. MUNNECKE, D.E. 1960. Hairy-root of field roses. Phytopathology 50 : 647-648 (abstr.)

149. MUNNECKE, D.E. CHANDLER, P.A. & STARR, M.P. 1963. Hairy-root (Agrobacterium rhizogenes) of field roses. Phytopathology. 53 : 788-799.

150. MURASHIGE, T. & SKOOG, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiologia Pl. 15 : 473-497.

151. NOEL, A.R.A. Callus formation and differentiation at an exposed cambial surface. Ann. Bot. 32 : 347-359.

152. NURSERY STOCK RESEARCH & EXTENSION ADVISORY COMMITTEE. (unpublished summary of ornamental plant research and experiments 1968-69) N.Z. Dept. of Agric. Wellington.

153. NURSERY STOCK RESEARCH & EXTENSION ADVISORY COMMITTEE. Minutes. N.Z. Dept. of Agric. Wellington.

154. NYLAND, G. & GOHEEN, A.C. 1964. Production of virus free material. Proc. int. Pl. Prop. Soc. 14 : 314.

155. PARSONS, L.C. & BEARDSLEY, R.E. 1968. Bacteriophage activity in homogenates of crown gall tissue. J. Virol. 2 : 651.

156. PATEL, M.K. 1926. An improved method of isolating Pseudomonas tumefaciens. Phytopathology. 16 : 577.

157. QUETIER, F. HUGUET, T. & GUILLÉ, E. 1969. Induction of crown gall : partial homology between tumor cell DNA, bacterial DNA and the G + C-rich DNA of stressed normal cells. Biochem. biophys. Res. Commun. 34 : 128-133.

158. RAABE, R.D. & WILHELM, S. 1966. Sources of infection in Verticillium wilt of rose. Phytopathology 56 : 123-127.

159. RAINBOW, A.F. 1966. Proliferation disease of roses. (unpublished report) Hort. Res. Centre - Levin.

160. RAINBOW, A.F. & POWELL, R.H. 1966. Report on investigations into nursery losses of newly-budded roses. Hort. Res. Centre - Levin (unpublished report).

161. RAINBOW, A.F. (unpublished experimental results) Hort. Res. Centre - Levin.

162. RAMASWAMY, S. & GARRETT, C.M.E. 1970. Virus like symptoms on cotyledons of Cucurbit plants caused by Pseudomonas syringae. van Hall. Pl. Path. 19 : 22-24.

163. RIKER, A.J. HILDEBRANDT, E.M. & IVANOFF, S.S. 1932. The development of crown gall, hairy-root, and wound overgrowth in glass cylinders. Phytopathology 22 : 179-189.

164. RIKER, A.J. & KEITT, G.W. 1926. Studies of crown gall and callus overgrowths on apple grafts. Phytopathology. 16 : 765-809.

165. ROBERTS, A.N. 1962. Scion bud failure in field grown roses. Proc. Am. Soc. hort. Sci. 80 : 605-614.

165a. ROBERTS, L. 1971. Importance of rose virus diseases. Gdnrs' Chron. 169 (12) : 6-7.

166. ROUFFA, A.S. & GUNCKEL, J.E. 1951. Leaf initiation, origin and pattern of pith development in the Rosaceae. Am. J. Bot. 38 : 301.

167. ROUSSAUX, J. KURKDJIAN, A. & BEARDSLEY, R.E. 1968. "Bacteriophages d' Agrobacterium tumefaciens" 'I' Isolation and characteristics. Annls. Inst. Pasteur. Paris 114 : 237-247.

168. SACHS, T. 1970. A control of bud growth by vascular tissue differentiation. Israel J. Bot. 19 : 484-98.

169. SACHS, T. & THIMANN, K.V. 1964. Release of lateral buds from apical dominance. Nature. Lond. 201 : 939-940.

170. SACHS, T. & THIMANN, K.V. 1967. The role of auxins and cytokinins in the release of apical buds from dominance. Am. J. Bot. 54 : 134-144.

172. SCHAEFFER, G.W. & SHARPE, F.T. Jr. 1969. Release of axillary bud inhibition with benzyladenine in tobacco. Bot. Gaz. 130 (2) : 107-110.

173. SCROTH, M.N. 1964. Induction of abnormal outgrowths in Datura by various agencies. Proc. Conf. Abnorm. growth Pl. Univ. Calif. pp 36-40.

174. SEN, M., PAL, T.K. & SEN, S.P. 1969. Inter-generic transformation between Rhizobium and Azotobacter. Antonie van. Leeuwenhoek 35 (4) : 533-540.

175. SHARP, W.R. & GUNCKEL, J.E. 1969. Physiological comparison of pith callus with crown gall and genetic tumors of Nicotiana glauca, N. langsdorffii and N. glauca X langsdorffii, grown in vitro "1" Tumor induction and proliferation. Pl. Physiol. 44 (7) : 1069-1072.

176. SHARPLES, A. & GUNNERY, H. 1933. Callus formation in Hibiscus rosa-sinensis L. and Hevea brasiliensis Mull. Arg. Ann. Bot. 47 : 827.

177. SKOK, J. 1967. Tumor and teratoma induction in tobacco plants by debudding. Pl. Physiol. 42 : 767-773.

178. SMITH, E.F. & TOWNSEND, C.O. 1907. Science. 25 : 671.

179. SOE, K. 1959. Anatomical studies of bark re-generation following scoring. J. Arnold Arbor. 40 : 260-267.

180. SORAVER, P. Vol. 1 Non-parasitic diseases. Manual of Plant Diseases (1914-22) 3rd Eng. Edit. Wilkes Barre: The Record Press, Paris.

181. STONIER, T. McSHARRY, J. & SPEITEL, T. 1967. J. Virol. 1 : 268.

182. STROUN, M. 1970. The natural release of nucleic acids from bacteria into plant cells and the transcription of host cell DNA. F.E.B.S. Lett. 8 : 349-352.

183. STROUN, M. ANKER, P. & AUDERSET, G. 1970. Natural release of nucleic acids from bacteria into plant cells. Nature. Lond. 227 : 607-608.

184. STROUN, M. ANKER, P. GAHAN, P. ROSSIER, A. & GREPPIN, H. 1971. Agrobacterium tumefaciens ribonucleic acid synthesis in tomato cells and crown gall induction. J. Bact. 106 (2) : 634-639.

185. STROUN, M. GAHAN, P. & SARID, S. 1969. "Agrobacterium tumefaciens RNA in non-tumorous tomato cells". Biochem. biophy. Res. Commun. 37 (4) : 652-657.

186. SYLWESTER, E.P. & COUNTRYMAN, M.C. 1933. A comparative histological study of crown gall and wound callus on apple. Am. J. Bot. 20 : 328-340.

187. TAYLOR, C.E. THOMAS, P.R. & CONVERSE, R.H. 1966. An outbreak of arabis mosaic virus and Xiphinema diversicaudatum in Scotland. Pl. Path. 15 : 170-174.

188. TEGLEY, J.R. WITHAM, F.H. & KRASNUK, M. 1971. Chromatographic analysis of a cytokinin from tissue cultures of crown gall. Pl. Physiol. 47 : 581-585.

189. THIMANN, K.V. & SACHS, T. 1966. The role of cytokinins in the "fasciation" disease caused by Corynebacterium fascians. Am. J. Bot. 53 : 731-739.

190. TOURNEUR, J. & MOREL, G. 1970. Sur la présence de Phages dans les tissus de "Crown Gall" cultivés in vitro. C.R. Acad. Sci. Paris. 270 D (23) : 2810.

191. TRAYLOR, J.A. WAGNON, H.K. & WILLIAMS, H.E. 1971. Rose spring dwarf, a graft transmissible disease. Pl. Dis. Reptr. 55 (4) : 294.

192. TULECKE, W. TAGGART, R. & COLAVITO, L. 1965. Continuous cultures of higher plant cells in liquid media. Contr. Boyce Thompson Inst. Pl. Res. 23 : 33-46.

193. WAGNER, D.F. 1969. Ultrastructure of the bud graft union in Malus. Diss. Abstr. B. 29 : 4556.

194. WARDLAW, C.W. 1965. The organisation of the shoot apex. Hand. der. PflPhysiol. 15 (1) : 966-1068.

195. WARREN-WILSON, J. & WARREN-WILSON, P.M. 1961. The position of regenerating cambia - A new hypothesis. New. Phytol. 60 : 63-73.

196. WEINSTEIN, L.H. TULECKE, W. NICKELL, L.G. & LAURENCOT, H.J. Jr. 1962. Biochemical and physiological studies of tissue cultures and

the plant parts from which they are derived III Pauls Scarlet Rose. Contr. Boyce Thompson Inst. Pl. Res. 21 : 371-386.

197. WHITE, P.R. 1943. A handbook of plant tissue culture. Jaques Cottell Press, Lancaster P.A.

198. WHITE, P.R. 1963. Culti. of Animal & Plant Cells 2nd Edn. Ronald Press Co. N.Y.

199. WHITE, P.R. & BRAUN, A.C. 1941. Crown gall production by bacteria free tumor tissue. Science. 94 : 239.

200. YARWOOD, C.E. RESCONICH, E.C. ARK, P.A. SCHLEGEL, D.E. & SMITH, K.M. 1961. So called beet latent virus is a bacterium. Pl. Dis. Reptr. 45 : 85-89.

201. ZIMMERMAN, P.W. HITCHCOCK, A.E. & CROCKER, W. 1931. The effect of ethylene and illuminating gas on roses. Contr. Boyce Thompson Inst. Pl. Res. 3 : 459-481.

FIGURES.

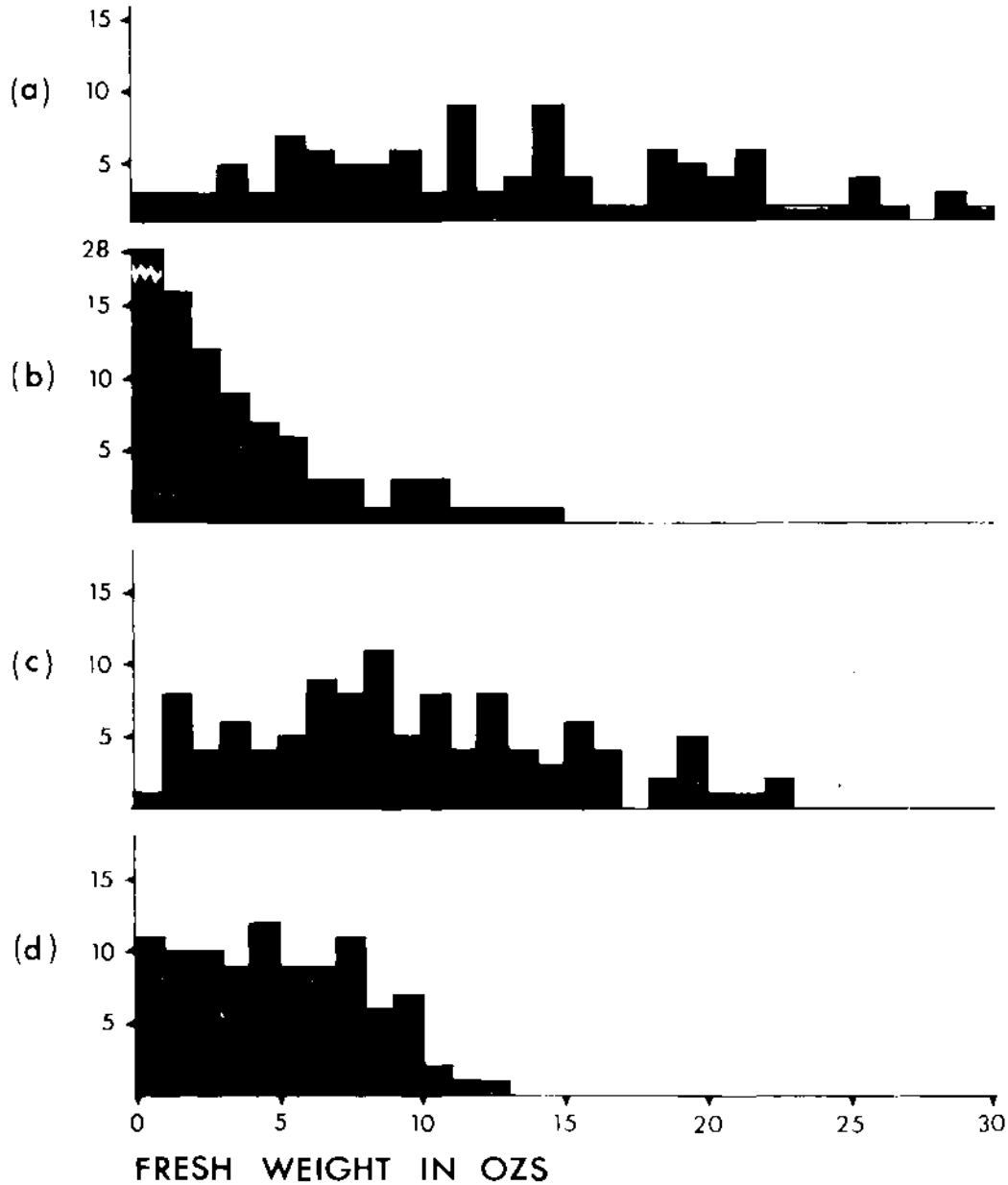

Fig. 1. Histograms showing distribution of weight classes in populations of:-

(a) stock plants from cuttings of seedling Rosa multiflora.

(b) stock plants from a commercial Rosa multiflora clone.

(c) resultant plants from budding on stocks as in (a).

(d) resultant plants from budding on stocks as in (b).

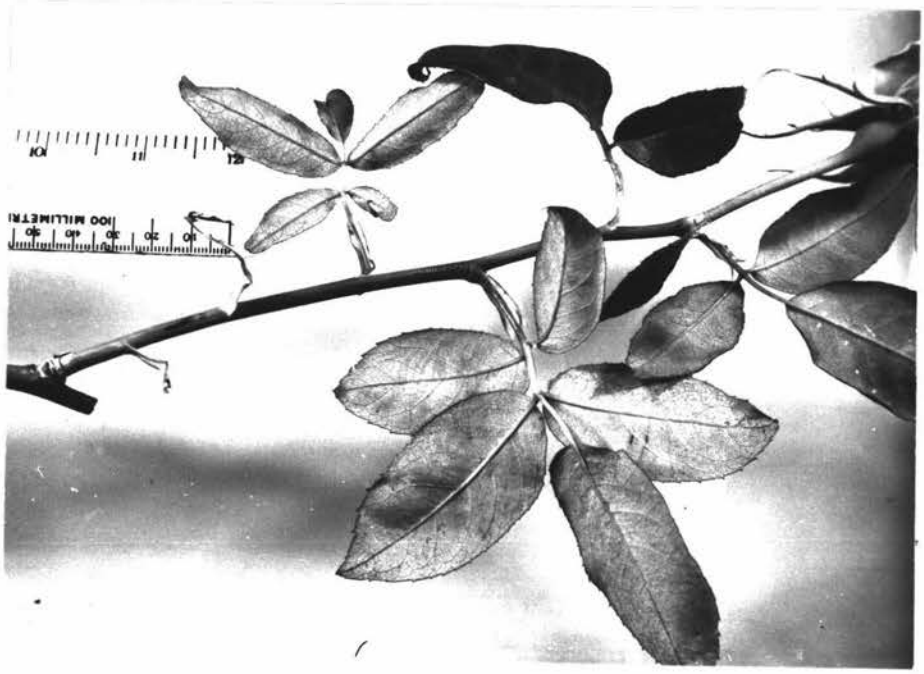

Fig. 2. A small normal shoot of a rose cultivar showing 5-leaflet leaf with budscale to leaf transitional series below and leaf to flower bract transitional series above.

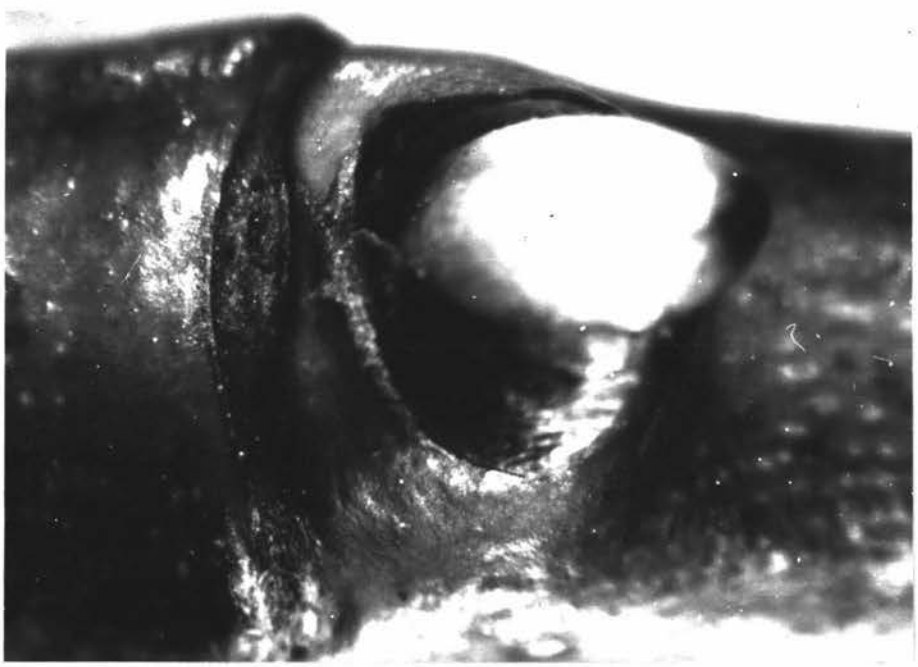

Fig. 3. Axillary bud from axil of fully developed leaf.

Fig. 4. Longditudinal section, transverse to main stem axis, through axillary bud showing clasping cataphylls and one of the two lateral secondary buds under one of the first pair of bud scales.

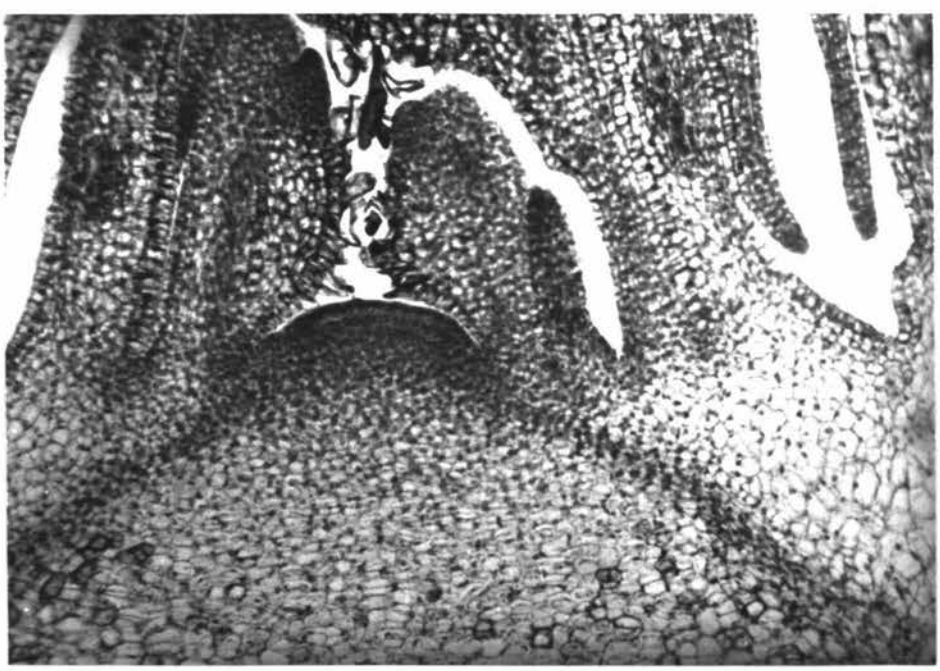

Fig. 5. Longditudinal section, transverse to main stem axis through apical dome of axillary bud and inner cataphylls with leaflet primordia.

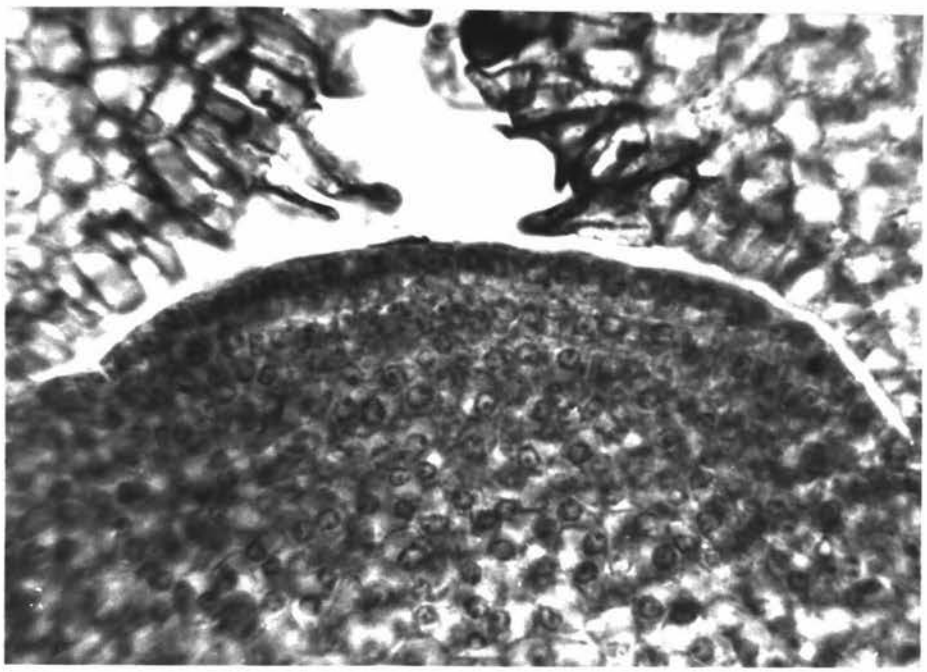

Fig. 6. Apical dome of axillary bud showing tunica layers and corpus.

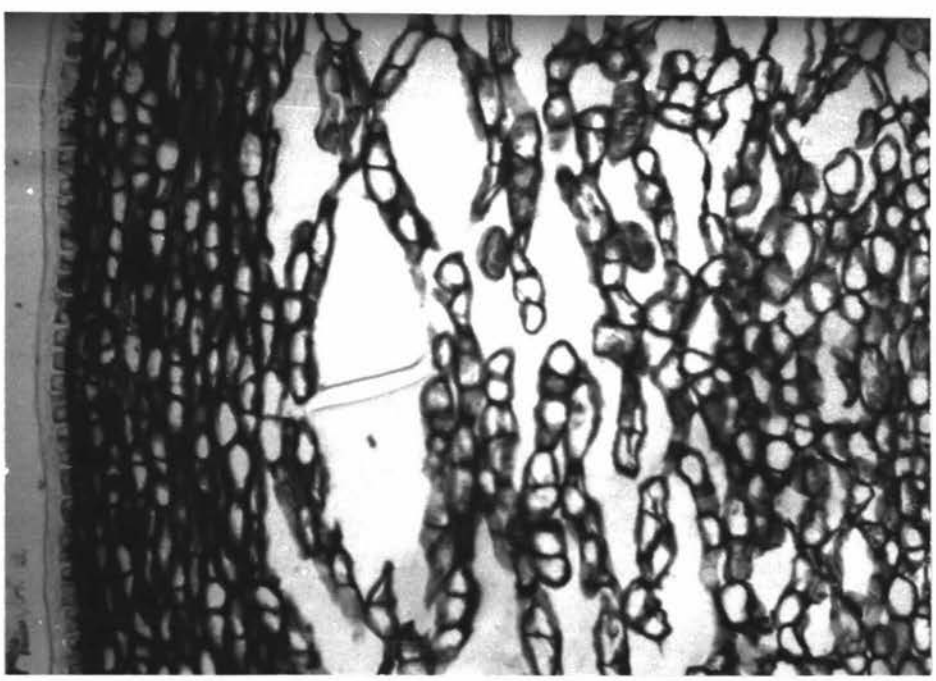

Fig. 7. Aerenchymatous tissue in stem at side of axillary bud.

Fig. 8. Typical proliferation symptoms from grafted bud with leaf from adjacent plant which had developed normally.

Fig. 9. Multiple proliferated shoots arising from normally undeveloped bud primordia of scion bud.

Fig. 10. First growth at top of young Rosa multiflora stock cuttings. Left: showing pronounced symptoms indicating galling at proximal end of cutting. Right: showing growth from normal ungalled cutting.

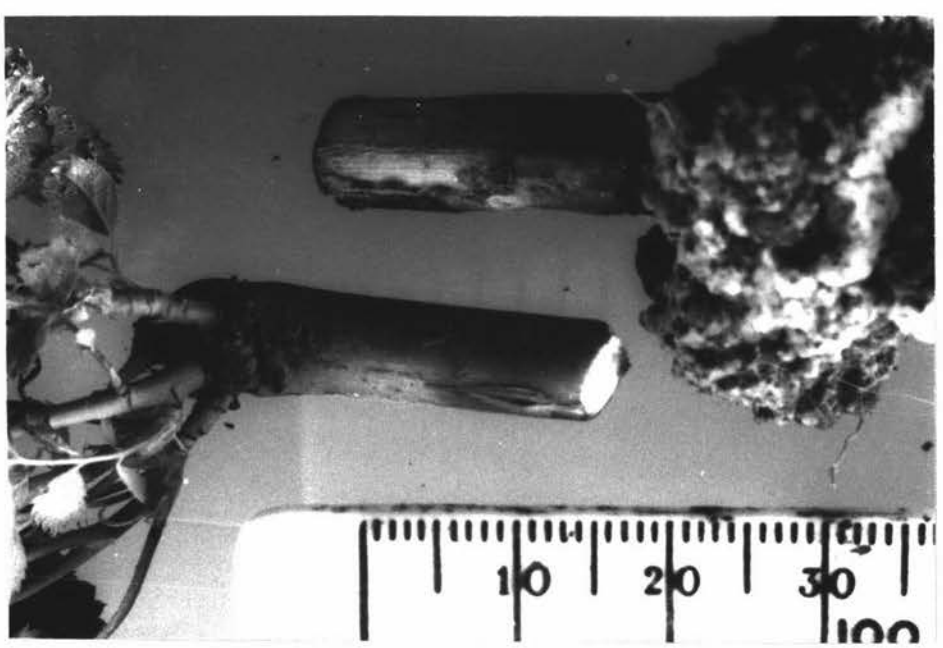

Fig. 11. Distal and proximal ends of the cutting shown in Fig. 10, left, showing extent of galling with pronounced top symptoms.

Fig. 12. Top of young stock cutting with first stock growth showing less pronounced symptoms consequent on basal galling. Slice removed shows vascular swellings.

Fig. 13. A selection of young Rosa multiflora stocks showing decrease in top growth with increase in galling.

Fig. 14. Close up of the base of the four heaviest galled stocks to the right in Fig. 13.

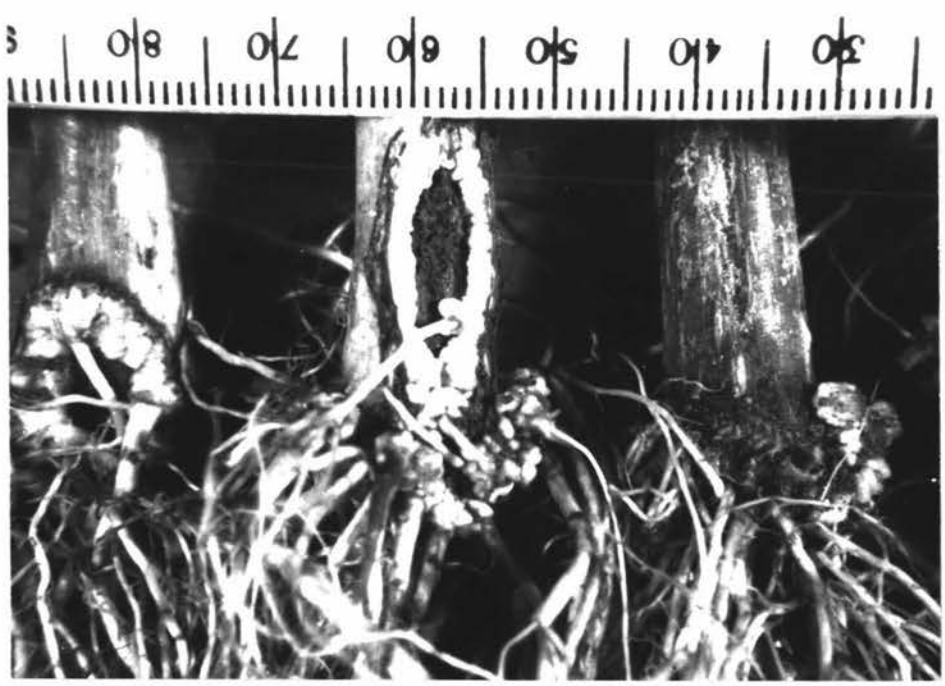

Fig. 15. Close up of the base of the four least galled stocks to the left in Fig. 13.

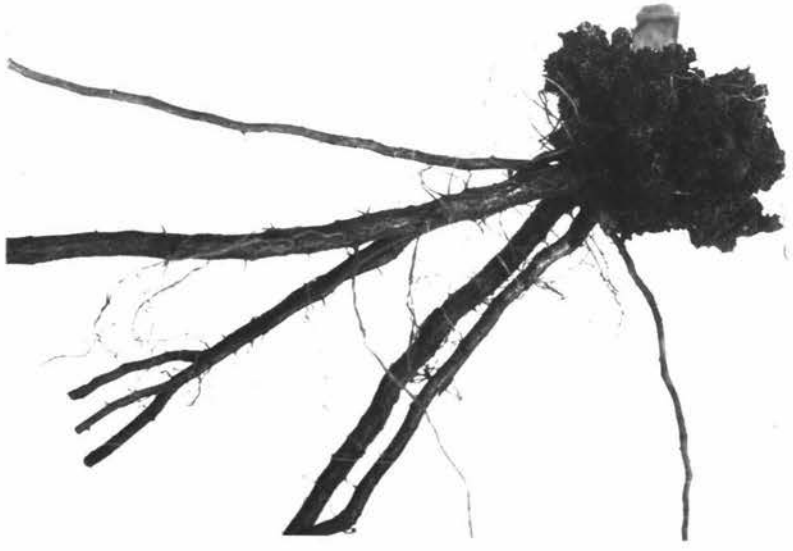

Fig. 16. Mature rose roots from below showing severe galling and distribution of roots reduced to $\frac{1}{4}$ circle around stem base. Recorded as 1. in experimental (Appendix G.).

Fig. 17. Mature rose roots from below showing slight galling and distribution of roots reduced to $\frac{3}{4}$ circle around stem base. Recorded as 3. in experimental (Appendix G.).

Fig. 18. Point of excision of the stock top (photo taken from above) showing excessive callus. The original grafted bud at the base of the proliferated shoots also shows swelling.

Fig. 19. Galling behind bud shield forcing it away from the stock and preventing graft union.

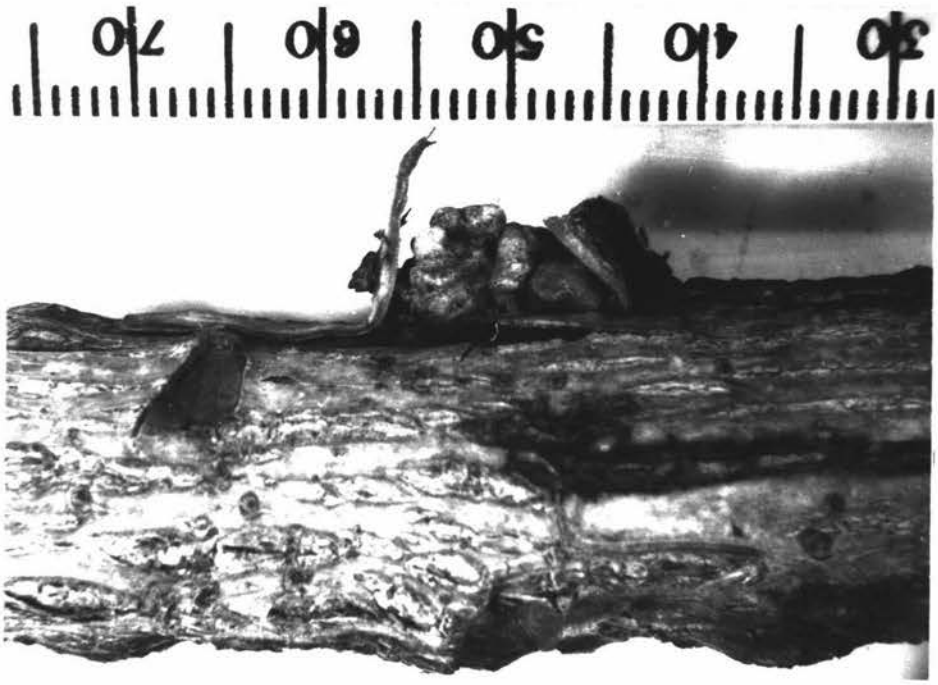

Fig. 20. Galling behind part of bud shield with the remainder of bud shield forming a normal union.

Fig. 21. Rosette symptom and die back on old wood with a young apparently normal shoot from the base of the plant.

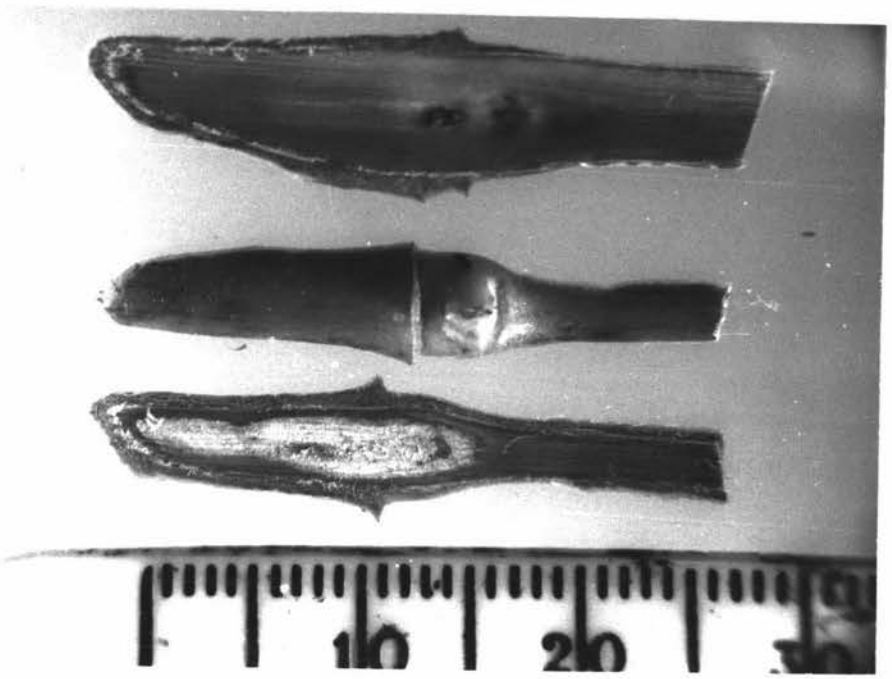

Fig. 22. Bud shields as used for bud grafting. Upper: back view with wood removed as used by Buck, (39). Centre: from the front. Lower: back view with wood in as generally used in New Zealand.

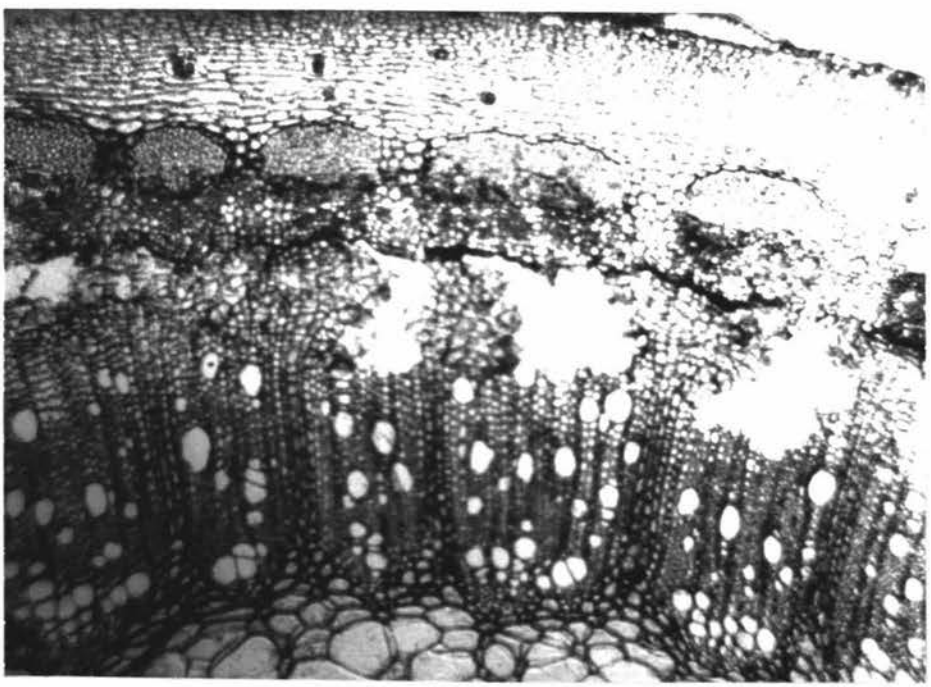

Fig. 23. Callus strands from xylem rays making contact with bud shield which has had wood removed as described by Buck (39).

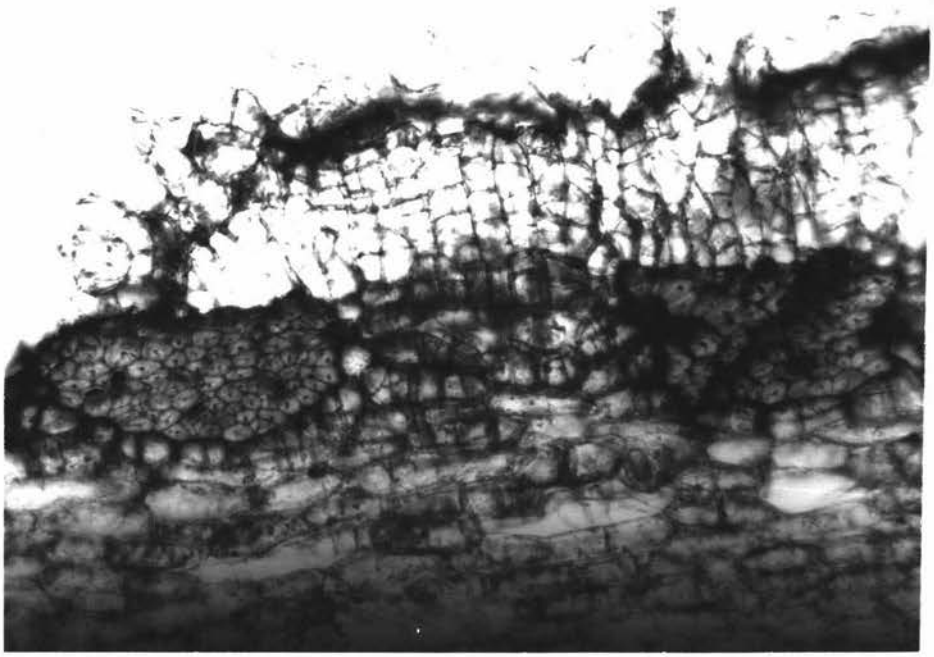

Fig. 24. Callus pad with radially arranged columns of cells behind necrotic plate. Large celled strands emerging above necrotic plate.

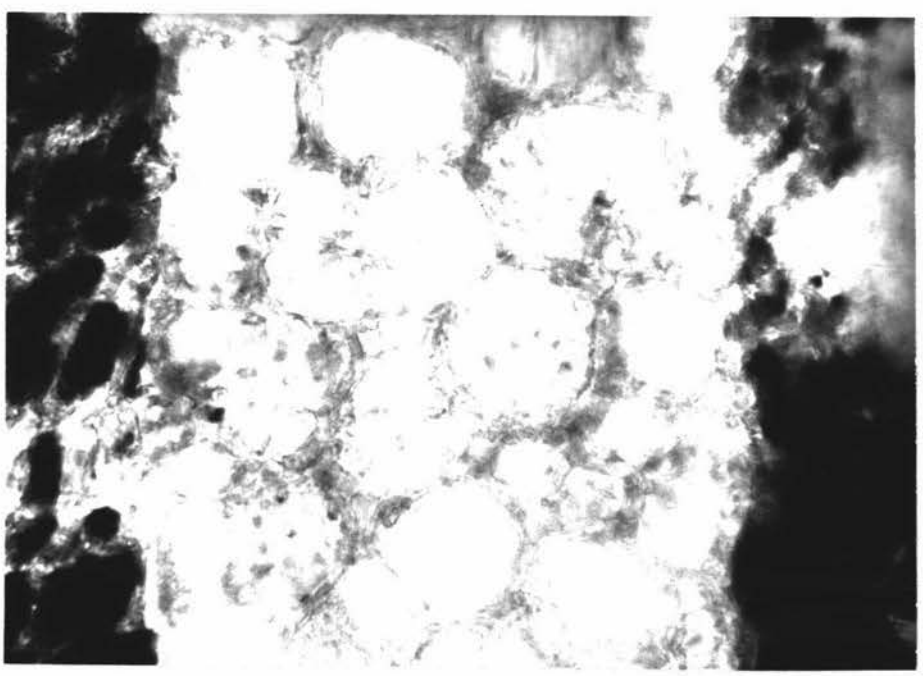

Fig. 25. Randomly arranged, abnormal, graft union callus starting to show pits as tissue matures.

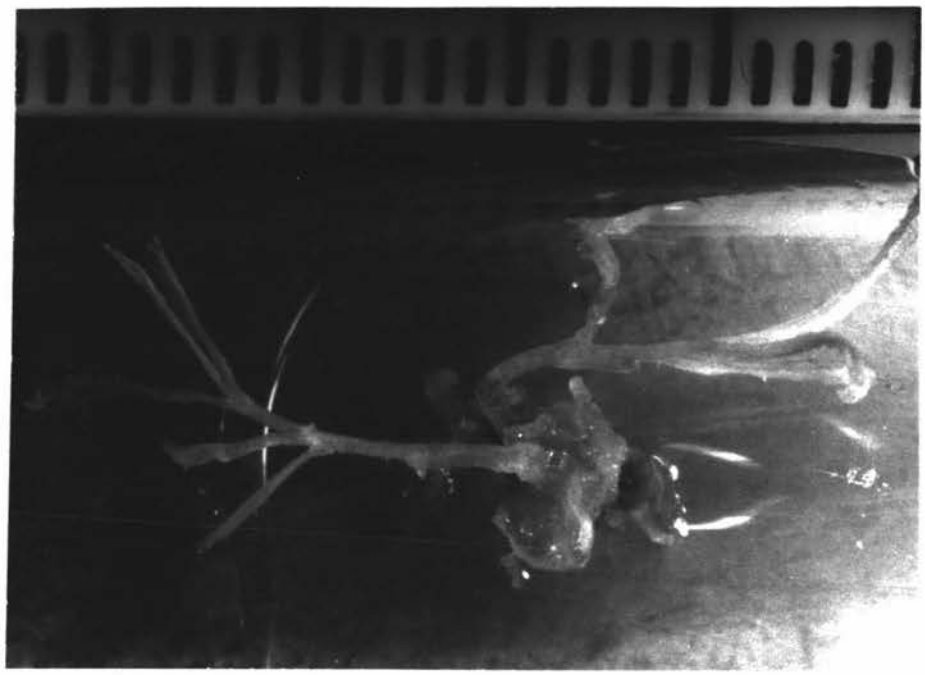

Fig. 26. Culture of meristem of axillary bud of Rosa multiflora on agar slope with basal medium plus benzyl adenine (2×10^{-7}M) and gibberellic acid (10^{-5}M).

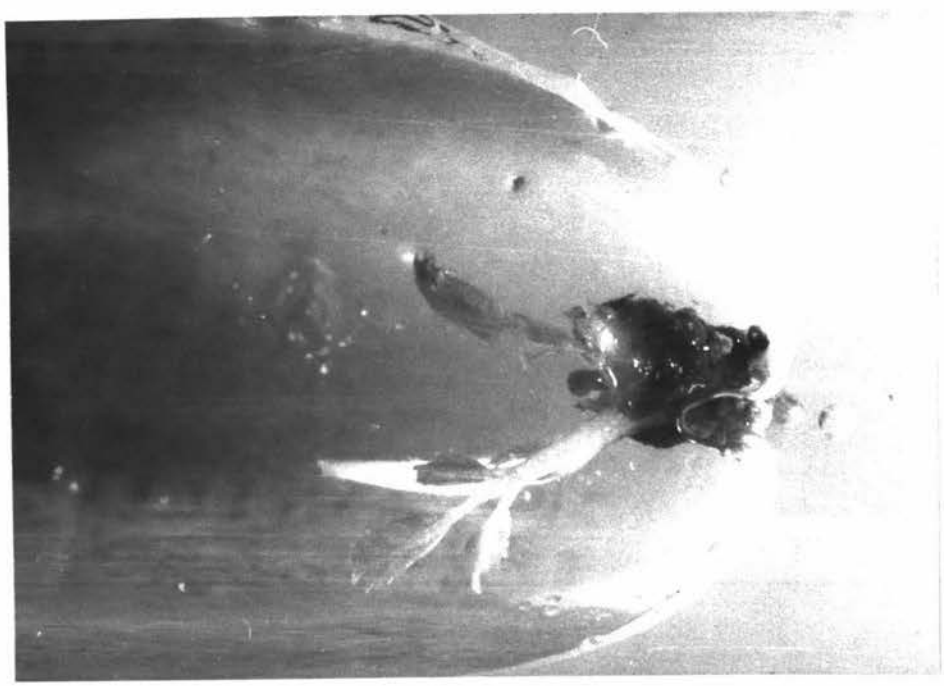

Fig. 27. Culture of meristem of axillary bud of Rosa multiflora on agar slope with basal medium plus benzyl adenine (2×10^{-7}M) and gibberellic acid (10^{-5}M) as for Fig. 26 with p-chlorophenoxyisobutyric acid (10^{-6}M) added.

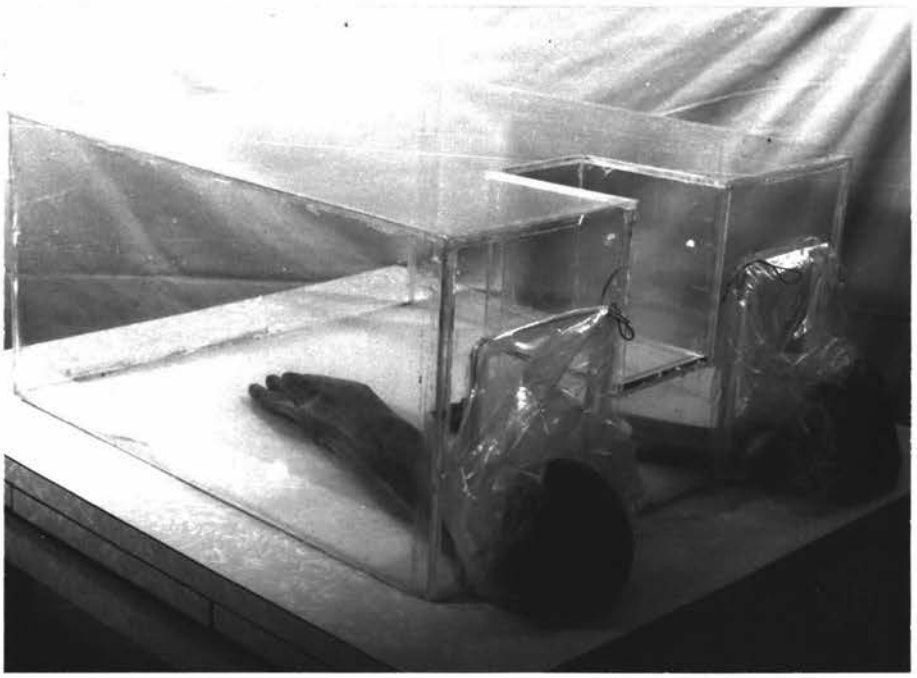

Fig. 28. Perspex cabinet constructed for sterile dissection of apical meristems showing arm ports and central sealed recess with plate glass bottom for use of foot-focusing dissecting microscope placed external to the cabinet but focusing within it.