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UPOV

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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

TECHNICAL WORKING PARTY FOR FRUIT CROPS

Twenty-sixth Session
Canterbury, United Kingdom, September 11 to 15, 1995

REPORT

adopted by the Working Party

Opening of the Session

1. The twenty-sixth session of the Technical Working Party for Fruit Crops (hereinafter referred to as "the Working Party") was held at Canterbury, United Kingdom, from September 11 to 15, 1995. The list of participants is given in Annex I to this report.

2. Mr. Guy Kerry from the PVRO Office in Cambridge and Mr. Peter Dodd from the Wye College welcomed the participants to Canterbury, United Kingdom. The session was opened by Mrs. Elise Buitendag (South Africa), Chairman of the Working Party.

Adoption of the Agenda

3. The Working Party adopted the agenda of its twenty-sixth session which is reproduced in document TWF/26/1, after having agreed to discuss under item 5 also other methods of image analysis. Due to the time spent on items 1 to 13, time was only available to discuss the Working Paper on revised Test Guidelines for Pear. The discussions took place partly in a subgroup, which reported to the main meeting and partly in the main meeting.

Short Reports on New Developments in the Member States in Plant Variety Protection for Fruit Species

4. The Working Party received short reports from some of the experts on recent developments in their countries. The experts reported that, in general, applications for fruit varieties were limited and would only cover a small part of the total applications in most member States. The most important crops by far would be apple and strawberry, followed by cherry, citrus, grape, kiwifruit, peach and pear. In some countries (France, Hungary, South Africa), varieties of all or certain fruit species needed to be included in a national list before they could be marketed. In others the inclusion was only necessary for the selling of certified plant material (Spain). As fees were collected for tests in Hungary, there was less incentive to apply for breeders' rights. In Norway, which had become a member State of UPOV in 1993, 100 applications have been received so far for only a few fruit varieties (no apples or pears, and only two for strawberries). About 10 grants have been issued, mainly for ornamental varieties. Norway is now applying the system to all species and it would try to rely heavily on the purchase of reports from other member States. In Chile, most fruit varieties would be of USA origin. In Italy, regionalization of government authorities had at that moment created a situation where future testing was left still undecided. In South Africa, sometimes, rights were only applied for in order to prevent marketing of the variety or other close varieties, without the applicant making efforts to market the variety himself. In several countries apple mutants would be a problem and too many applications were either withdrawn or would have to be rejected. A more detailed report from France and Hungary is attached to the report as Annexes II and III.

5. The expert from the European Union (EU) informed the Working Party of the following action by the European Commission to implement Council Directive 92/34/EEC of April 28, 1992, on the marketing of fruit plant propagating material (including plants intended for fruit production): (a) Commission Directive 93/79/EEC setting up additional implementing provisions for lists of varieties of fruit plant propagating material and fruit plants, as kept by the suppliers; (b) Comparative trials carried out on strawberry propagating material.

6. On that occasion, the Working Party also discussed the different procedures in the individual member States with respect to the period between the date of application and the granting of rights. Some member States granted protection during that period but under different conditions. In the United Kingdom, the applicant could be granted, on request, protective direction as long as he abstained from marketing the varieties; in other States the applicant received protection as of the date of application, would be able to sell his variety immediately after application and collect royalties. Because of the short life of many varieties the possibility of immediate sales was very important. In other States, even though the variety was only protected after the grant of rights, the applicant could already ask in advance for license fees. He would obtain them mainly because of the pressure to keep commercial relations with him because of his other protected varieties. It was left open whether the license fees paid could be recovered if the candidate variety had been rejected.

7. In order to get a clear picture of the different practices and rules in the individual member States on provisional protection, but also on whether a compulsory or facultative variety list existed and the requirements for inclusion in such a list, the Working Party established a list of questions for a questionnaire to be circulated inside its group.

Important Decisions Taken During the Last Sessions of the Technical Working Party, the Technical Committee and the Technical Working Party on Automation and Computer Programs

8. Mr. M.-H. Thiele-Wittig gave a brief report on the main items discussed during the previous session of the Technical Committee and referred participants needing further details to the full report reproduced in document TC/31/6.

9. Electrophoretic Characteristics: The Working Party noted that the majority of the Technical Committee had been of the view that it was not possible to establish distinctness solely on the basis of a difference found in a characteristic derived by using electrophoresis, and that such characteristics should therefore only be used as a complement to other differences in morphological or physiological characteristics. It noted that the Technical Committee had decided to take these characteristics out of the main text of the Test Guidelines and to place them in an Annex, thereby creating a special category of characteristic and that the Test Guidelines should state that these characteristics were considered useful but that they might not be sufficient on their own to establish distinctness and thus should not be used as a routine characteristic, but at the request, or with the agreement, of the applicant for the candidate variety.

10. Working Procedure for Establishing Test Guidelines: The Working Party noted that the Technical Committee had recommended that in the preparation of new or revised Test Guidelines there should be at least two responsible experts/countries and not just one as at present, especially in small crops where large subgroups were not justified, so as to ensure that work would continue even if a responsible expert was prevented from attending a given session of a Working Party. It had also agreed that in future new drafts would be presented to the Editorial Committee at the same time as they were sent to the professional organizations for comments and that the Editorial Committee would not limit itself to highlighting linguistic discrepancies but would also ensure that UPOV concepts were maintained in the documents and would highlight where this was not the case, and would propose, as far as possible, solutions for any such shortcomings.

11. Request for Photos in the Technical Questionnaire: The Working Party noted that the Technical Committee had supported the request for a representative color photo of the candidate variety in the Technical Questionnaire, however, limiting the obligation to ornamental species only. It saw no reason why the same rules should not also apply to fruit species and asked the Technical Committee to extend that rule also to fruit species.

12. Genetically Modified Organism (GMO) Varieties: The Working Party noted the position of the Technical Committee vis-à-vis the handling of GMO varieties. It considered it, however, important to know when a candidate variety was a GMO. It therefore proposed to include in the Technical Questionnaire under paragraph 4 or paragraph 7 a sentence which could have the same wording as proposed by the Technical Working Party for Ornamental Plants and Forest Trees (TWO), namely: "The candidate variety represents a Genetically Modified Organism [ ] Yes, [ ] No." It asked the Technical Committee to find the final wording and to discuss whether a definition on what was considered a GMO was also necessary to be included as it had been done in the Technical Questionnaire of the EU PBR Office.

13. Example Varieties: The Working Party noted that the Technical Committee had asked all Technical Working Parties to rediscuss the handling of example varieties and report to it during its next session. The Working Party also noted that the Committee had agreed that under certain conditions, as mentioned for Citrus, it was not necessary to give example varieties for each state of expression of each characteristic.

14. Definition of Off-Type: The Working Party noted that the Technical Committee had agreed to the conclusion of the TWO that each plant which showed a mutation in parts of its organs was considered to be an off-type. For fruit species, the Working Party could not follow that proposal as it would lead to a rejection of most applications. In fruit species the situation was different. An apple tree could not be considered an off-type if only one leaf of the tree or one apple was an off-type, which was impossible. On the other hand, if a whole branch was an off-type, the tree had to be considered to be an off-type. The problem was to define the border, as from when it had to be considered an off-type. Some experts proposed as border line whether the mutated organ could be used to produce another variety or whether the mutation could be perpetuated. However, with the help of new methods and tissue culture, nowadays parts too small could already fulfill that requirement.

15. The Working Party finally agreed to propose to the Technical Committee that it amend the definition of the TWO whereby each plant which showed a mutation in parts of its organs was considered an off-type, so that not all mutations but only "significant" mutations of part of an organ should be considered an off-type.

16. Cooperation with Breeders in the Testing of Varieties: The Working Party noted that the Technical Committee had asked that the survey on the involvement of breeders or applicants in the testing of varieties be repeated so as to also cover non-agricultural species in order to have full details of the testing systems of the individual member States. A new questionnaire (U2268) had been circulated for completion, the anwers to which were included in document TC/32/4. The experts from Canada and Japan explained in further detail the involvement of the applicant in their testing. In both countries the applicant would grow the plants and the examiner from the office would visit the trials at a time when most of the characteristics could be observed. In both cases, the examiner would observe all characteristics possible at the given time. In Canada, the applicant would also have to make the same observations and the remaining ones which then would be compared by the office with the observations of the examiner. If both agree, a decision would be taken and published within a period of 6 months for objections. The office would also check whether the comparable varieties were correct, and if not, ask for further testing. The testing of uniformity would be left to the applicant. In Japan, in addition to the observations of the examiner, the applicant would have only to test the characteristics that had not been possible to be observed during the visit. The Japanese office would have already at the start given the applicant detailed descriptions of the layout and on similar varieties. In case of doubt, or when a candidate variety was too close to a candidate of another applicant, the office would do its own tests in one of the 11 stations or in an institute or a regional authority (especially for rice). In addition to these growing tests performed by the office itself, a third possibility existed for varieties bred by public institutes where sufficient data would be available in order to rely on a written report.

17. The experts from Hungary and Norway reported that for fruit species the competent authority did not have its own collections and would have to rely on the plants grown by the applicant. All observations would, however, be made by the competent authorities even if it required several visits to the trial grounds. In the case of Chile, the competent authority would rely on plants grown by the applicant, if he had a collection of varieties. In Israel, it was also possible that the plants were grown by the applicant and the expert would observe them there. In New Zealand, for fruit species, the observations on the premises of the applicant had been abolished and all fruit applications were now grown and tested centrally.

UPOV Documents in Electronic Form

18. The Working Party noted that the Technical Committee had requested that a survey be made in order to inquire who would be interested in documents in electronic form and for which purpose it would be needed, before asking the Office of UPOV to keep the electronic version of documents in full agreement with the printed versions. Having discussed different possibilities of availability on diskettes, on-line, etc., and what kind of documents should be made available (all Test Guidelines, all reports of meetings, all documents for meetings, etc.), it finally proposed to start with a firm but limited trial to place the report of the session and the agenda and, if possible, the same for all other Technical Working Parties and the Technical Committee on one diskette to be distributed to the TWF. The documents should be recorded in parallel in Word for Windows and in ASCII. Then, during its next session it would report on the use made of the diskette.

Color Observations

19. The experts from France, Germany, Hungary, Italy and the Netherlands reported on measurements of color which in most cases had been discontinued or reduced. The work had mainly been in the research stage but also in certain cases been used to support visually-observed differences. During a visit to the Brogdale Farm, the expert from the United Kingdom gave a detailed report on the studies of color measurements of apples made in the United Kingdom. With a Minolta CR-200 Chroma Meter, three measurements of solid color of 10 fruits per variety would be made and the L, a* and b* values recorded. It was not important to measure the exact color but only the relationship between two varieties which were compared for distinctness. Research on measurement will be continued and reported upon during the next session. More details on that project are reproduced in Annex V to this report. Several experts stated that emphasis had shifted from color measurements to image analysis.

20. Image Analysis: The Working Party noted that the Technical Committee had requested that a survey be carried out of what had already been done in the field of image analysis and what problems had been encountered with that tool in variety testing. Some delegates had warned that it may be difficult, especially for the system of breeders' testing, to make any characteristic mandatory which could only be observed with that tool. This should also apply to any other methods which breeders themselves may find difficult to apply not only before applying for breeders' rights but also in the maintenance of the variety after the granting of protection.

21. The Working Party also noted information on a research proposal for the European Communities written as a result of Circular U 2220, a Questionnaire on Image Analysis in Variety Testing. The project was submitted to the FAIR-program of the European Communities in March 1995 under the acronym VISOR. The objectives of the project were to:

(a) establish best practice guidelines in applying image analysis to testing for distinctness, uniformity and stability;

(b) develop computer systems which automate the production of scores for characteristics which are currently visually assessed;

(c) develop an image database system for plant varieties which can take an image of one variety and compare it with other images of varieties of the same species in order to identify the closest visual match.

22. The Working Party noted that the Technical Working Party for Ornamental Plants and Forest Trees (TWO) had reserved during its session the previous week a whole day on image analysis and would reserve half a day for that purpose during its next session. The questions under study were the replacement of measuring of characteristics, the development of new characteristics and the observation of colors. During its visit to the National Fruit Collections at Brogdale, Faversham, the experts from the United Kingdom explained in detail their project of measuring apples by image analysis through the scanning half of the profile of an apple and the calculation of 18 coordinates and the relative position of the coordinates to each other. By using this method it was planned to measure the whole apple collection in order to obtain comparable data on about 2,200 varieties. More details on that project are produced in Annex IV to this report.

23. The Working Party also noted information from New Zealand on research on image analysis to determine red color (an abstract which is reproduced in Annex VI), on digital imaging (an extract which is reproduced in Annex VII), and on the differentiation of apple sports by pollen ultrastructure (a summary which is reproduced in Annex VIII). It finally agreed to collect for its next session more detailed information on the use of research on image analysis. The expert from Germany offered to compile all information sent to him before the end of January 1996. The Office of UPOV was asked to send a reminder to all experts.

New Methods, Techniques and Equipment in the Examination of Varieties

24. The Working Party recalled the report on the second session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular (BMT), reproduced in document BMT/2/9, and that the next session of the Working Party would take place in the week following the present session. The expert from the Netherlands offered to attend that session and would report to the Working Party during its coming session. The Working Party recalled its position that more effort should be made to develop the observation of morphological characteristics using new tools, for example, image analysis. The observation of pollen and its surface might well be developed for the purposes of distinctness. Image

analysis might also speed up the number of other morphological observations. In clonal material DNA profiling would be of little help, as in mutations it was difficult to find differences in the profile compared to the original variety.

25. The Working Party objected to the term "fingerprinting" applied to DNA-profiling. A human fingerprint was unique, a DNA-profile, however, was not unique. The Working Party discussed the term "identification" compared to "distinctness." Identification meant tracing existing varieties. DNA-profiles may allow fast tracing of a given variety, e.g. when plant material falling under the scope of variety protection was imported.

26. The Working Party discussed whether the possibility of identification with DNA-profiles could also be used to facilitate the screening of varieties, e.g. for similar varieties to a candidate variety for their selection for the growing test. The Working Party noted that in certain crops similar varieties would be selected in that way, however, not in fruit crops. DNA-profiling would also not be used for grouping varieties. In order to get a better overview of what was happening with electrophoresis and DNA-profiling in the individual member States, the Working Party agreed to collect information on the use of these methods for identification, tracing of varieties and for screening them. The expert from the Netherlands will collect the information to be sent to him by all experts before the end of January 1996. The information should not be limited to fruit species only; information on other vegetatively propagated species would be welcome. The Office of UPOV was asked to send a reminder to all experts.

Bibliography of Published Papers on new Techniques

27. The Working Party noted document TWF/24/8 and new information collected by the expert from the United Kingdom and distributed on diskettes. It was mainly extracted from the Commonwealth Agriculture Bureau International in Oxford and also contained abstracts. Some further information received from member States was also included. The expert from the United Kingdom offered to remain as contact person for further updating of that list. Copies of that diskette could be requested from the Office of UPOV.

Statistical Methods

28. The Working Party noted that the Technical Committee will follow the study of the TWA together with the TWC on whether the COYD analysis developed for cross-fertilized species could also be applied to self-fertilized species. It stressed that it should be ensured that where statistical methods were changed there should be no abrupt change in the number of varieties rejected or accepted. In addition, the method should only be introduced for species where real problems of distinctness existed.

29. The Working Party noted an explanation given by the expert from Germany on document TWC/11/12 on the assessment of visually-observed characteristics. The document was based on the results of Pelargonium varieties over many years. In the beginning the Working Party were reluctant to follow all the conclusions of the document. It asked to be cautious with characteristics which were market oriented. All breeders would breed in that

characteristic for certain states of expression which would therefore lead to changes in the normal distribution of the states of a quantitative characteristic (e.g. breeding for very early and very late varieties) or would lead to most varieties being found in one or two states of a qualitative characteristic like color.

30. After further discussions the Working Party finally agreed that the method described in the document was very useful for the crop expert in helping him (i) to judge whether the number of states of expression used for each characteristic was justified or needed an amendment; (ii) to note which characteristics were correlated and could be reviewed with a view of possibly eliminating one of them, and (iii) to check whether the minimum distance applied was correct or should be adjusted. The Working Party insisted that the method should just help the crop expert but not force him into making changes. The final decision had to remain with the crop expert. The Working Party recommended that the method be considered at every revision of an existing Test Guidelines document, although in practice at present the same review of the existing characteristics took place automatically without the application of statistics.

Uniformity in Vegetatively Propagated and Self-Pollinated Varieties

31. The Working Party noted that the Technical Committee would further discuss the balance of the risks of wrongly rejecting a uniform variety as heterogeneous and of wrongly accepting a heterogeneous variety as uniform, as well as the influence of the sample size on these risks. It had asked that documents TWC/11/16 and TC/30/4 be revised and drafted in a language which could be more easily read and understood. It welcomed the intention of the TWC to redraft the documents on the COYD and COYU analyses and document TWC/11/16 on uniformity to make them more user-friendly.

32. The expert from the United Kingdom in the TWC gave to the Working Party a new introduction to document TWC/11/16 explaining the main aims of the document and the limitations with respect to small samples. Several experts stated that there was a clear difference between the method developed for self-pollinated species and the situation in vegetatively propagated varieties. While in the first case there was genetic variation between the individual plants, there was no genetic difference between the plants in the second case. Unfortunately, in UPOV both cases were placed into one single group in the General Introduction to Test Guidelines and thus also in document TWC/11/16. In addition, a high mutation rate in vegetatively propagated material was often not caused by the material itself but by the breeder or applicant who had not done his work properly and applied too early for protection.

33. Other experts wondered whether the fact that some plants had few flowers and others many would have to enter into the statistical calculations. The situation may also have to be looked at differently from characteristic to characteristic and it was not possible to apply one single population standard equally to all characteristics in the same way but certain characteristics could justify a different population standard in the same way, as inside a species different types of varieties could justify different population standards (e.g. apple rootstock varieties and fruit varieties, vegetatively propagated and seed propagated varieties).

34. While the Working Party accepted that in principle it was possible to judge characteristic by characteristic, that it was not, however, possible in practice and one single population standard should be selected for one species or at least for one group inside a species (e.g. all fruit varieties, all vegetatively propagated species). In principle the population standard should be 1 per cent with an acceptance probability of 95 per cent unless special reasons (e.g. a different source of the material) justified a different percentage (e.g. a high mutation rate).

35. The question was also considered to be connected with the definition of an off-type. As already stated above, it was not possible that any difference in the tree of an apple variety was considered to be an off-type. A tree, in general, showed many small differences and almost all leaves were slightly different. It was necessary to observe an overall view. The leaves on a tree were almost a population inside the tree and it was more the question of how much variation inside a tree was acceptable before one would speak of off-types.

36. In order to get a better understanding of the problem, the Working Party asked the expert from the United Kingdom to compile some data on mutations of apple varieties and submit them to his colleague in the TWC for calculations in order to inform all the experts in the TWC better and to make them more aware of the special situation.

37. The Chairman also explained that the fact that the application of the method in TWC/11/16 could lead to a high β-risk which was far from reality was partly explained by the fact that, of the total curve in question, only the first part was relevant. It was unlikely that the number of off-types would be higher than a very low number since the variety was normally derived from a single clone. In the relevant range of the curve, the β-risks would, however, not differ too much from that of a larger sample.

38. The Working Party also made it clear that while in agricultural varieties the variety had only to be uniform in characteristics routinely used for distinctness, in ornamental and fruit varieties it was not possible to ignore off-types in characteristics which so far had not been used for distinctness purposes. In its area of crops uniformity would thus be looked at differently.

39. The Working Party finally appreciated the presence of the expert from the TWC and its explanations on document TWC/11/16. The dialogue now taking place between the crop working parties and the TWC was very much appreciated. They now tried to understand each other's problems, which was a sound basis for finding solutions to the outstanding problems.

UPOV Central Computerized Database

40. The Working Party noted the latest stage of preparation of the UPOV central computerized database on CD-ROM as set forth in Circular U 2229 dated February 24, 1995. The Office of UPOV had invited all of its member States to submit data for the envisaged UPOV-ROM Demonstration Disc by April 15, 1995. It had received data from 15 States (Argentina, Austria, Canada, Denmark, France, Germany, Hungary, Israel, Japan, New Zealand, Netherlands, Spain, Sweden, United Kingdom, United States of America (PVPO and PTO)). The Office of UPOV, with the help of experts from WIPO, had checked the data

received and had requested, if necessary and possible, corrections from some of the countries. Afterwards, all data were submitted to JOUVE for the preparation of the above-mentioned UPOV-ROM Demonstration Disc. The Working Party also noted Circular U 2277 containing a list of open questions on the UPOV-ROM Demonstration Disk. All experts were invited to send their comments or proposed answers to the Office of UPOV. After having corrected the data it was expected that the Demonstration Disc would be sent to the member States, if possible, during the week of the meeting.

41. The fruit experts appreciated the establishment of the UPOV-ROM as they urgently needed such a database. They expressed the hope that all member States would eventually participate in the database so that all varieties might be covered. They noted that details of distribution outside the circle of the competent authorities had not yet been fixed. The Demonstration Disc itself, however, was also sent to a selected number of non member States and governmental and non governmental organizations.

Characteristics on Disease Resistance

42. The Working Party noted that the Technical Committee had agreed that disease resistance and tolerance characteristics were acceptable for the establishing of distinctness if they fulfilled the same requirements for acceptance as any other characteristic. It was of importance that any such characteristic was well defined and that an accepted, standardized method existed for its evaluation. Although the Technical Committee accepted the inclusion with an asterisk of resistance characteristics in the Test Guidelines adopted during its session, as well as the inclusion of a resistance characteristic with quantitative expressions from "absent or very weak" to "very strong," future examples should be decided on a disease-by-disease and species-by-species basis. The Technical Committee also had agreed on the following definition:

Resistance: The ability of a variety or of a mono-specific population to limit the activities of a given pest or pathogen throughout the whole or a part of a growing cycle. Several resistance levels may generally be defined.

Susceptibility: Susceptibility corresponds to a zero-resistance level of a variety or population with respect to a given pest or pathogen.

Tolerance: Ability of a variety or population to tolerate the development of a pest or pathogen whilst displaying disorders that are without serious consequences for their growth, appearance or yield.

43. The Working Party noted the definitions and would in future follow them. Some experts stated that in the case of the above-mentioned resistance in Japanese Pear a major gene was involved. The situation would be more difficult in polygenetic controlled resistance. It was necessary to know the genetic background exactly before accepting such characteristics.

List of Species of Which Practical Technical Knowledge Has Been Acquired

44. The Working Party noted that the Technical Committee had requested that the present document TWO/27/13 comprising a list of species of ornamental plants tested in the UPOV member States be extended to cover all species of which practical knowledge has been acquired in the member States and that a new questionnaire (U 2229) had been sent out and the answers to that questionnaire had been included in document TWO/28/10.

45. The Working Party appreciated the paper and asked its members to check the information in the document or in a new version to be prepared which would cover also information from Spain received after the printing of the document. The Working Party proposed that this document should also be made available in electronic form.

Discussions on Working Papers on Test Guidelines

Standardization of Certain Terminology and Specifications

46. The Working Party discussed at length possibilities to standardize the wording used in the different Test Guidelines and also to harmonize its approach more to similar situations in different species. Several decisions reached for the Test Guidelines for Apple were thus, where appropriate, immediately also applied to the other Test Guidelines discussed. In order to even improve the standardization of certain terms more, especially for characteristics of shape, the Chairman would try to prepare some proposals for the next session. The Working Party was also reminded of document TC/27/5 prepared by the Office of UPOV some years ago which already proposes harmonized states of expression for numerous key words. The Working Party was also requested to consider harmonizing the approach to certain characteristics more difficult to assess as pubescence, acidity, sweetness and juiciness.

Final Discussions on Draft Test Guidelines

Draft Test Guidelines for Apple (Revision)

47. The Working Party noted document TG/14/6(proj.) and comments from Japan and Spain raised during the session and made only the following main changes in that document:

(i) Subject of these Guidelines: To have the second sentence deleted and the third sentence transferred to IV(2) after having replaced the word "figure" by "number of observations."

(ii) Material Required: To have in paragraph 1 before the words "10 trees" the sentence included reading: "If accepted by the competent authority the applicant could submit:" and to have the last sentence deleted. To have in addition the words "vegetatively propagated" inserted before the mentioned rootstocks. This change to apply to all fruit tree varieties where applicable. In paragraph 2 and in the Technical Questionnaire to have the words "meristem culture" replaced by "*in vitro* propagation." The last mentioned change to apply to all Test Guidelines.

(iii) Methods and Observations: To have paragraph 1 in these Test Guidelines as well as in all other Test Guidelines replaced by the standard paragraph on the population standard, the acceptance probability and the number of off-types admissible. The acceptance probability would be 95 per cent for all fruit species. For apples the other figures would be for hybrid varieties 1 per cent = 0 off-types in 5 plants, for mutants 2 per cent = 1 off-type in 10 plants. In paragraph 2 the number of observations to be: "five plants for hybrid varieties, 10 plants for mutants."

(iv) Table of Characteristics:

Characteristics

3 To have the bracketed addition "Columnar types excluded"

6 To have the additional states "very short (Wijcik), very long"

8 To have the example varieties corrected as follows: "1 (none), 2 (Herrenhuter), 3 (Gravensteiner), 4 (Sylvia), 5 (Kidd's Orange Red), 6 (Ratfubin)"

9 To have "Jonathan" deleted

13 To have "Golden Delicious" replaced by "Jonagold"

15 To have "Worcester Pearmain" replaced by "Jonagold"

19 To have the second state read "in middle"

20 To have the example varieties for states 9 and 10 exchanged, and the word "oblate" replaced by "obloid"; the states of expression will require further study but should not hold up the adoption of the Test Guidelines.

21 To have "prominence of" deleted and "Bloody Ploughman" corrected

25 To have "Ŝampion" corrected

28 To have "Boskoop" corrected

32 To be deleted

33 To have the states "absent or very weak (Golden Delicious, 1), weak (2), strong (McIntosh, Florina, 3)"

34 To have the states "absent or very weak (Gloster, 1), weak (2), strong (Jonagold, 3)"

35 to 39 To have the words "of skin" deleted

36 To receive an asterisk, to have the first "color" replaced by "hue" and to have an additional state "pink (Coripp's Pink)" after "red"

37 To have the first "color" replaced by "hue"

39 To have the states "only solid flush (1), only striped (2), solid flush and striped (3), mottled (4), washed out (faded) (5)"

44 To have "MacIntosh" deleted

48 To read: "Time of maturity for consumption"

(v) Technical Questionnaire: To have under paragraph 4 a sentence requesting the applicant to state whether his variety is a GMO variety or not and under paragraph 7 a sentence requesting the applicant to add a representative color photo of the candidate variety. These two additions to be made in all Test Guidelines.

Draft Test Guidelines for Strawberry (Revision)

48. The Working Party noted document TG/22/7(proj.), proposals prepared by experts from Spain, comments received during the session and the following main changes in that document:

(i) Subject of these Guidelines: To apply to all vegetatively propagated varieties of "*Fragaria* L."

(ii) Material Required: The material recommended to be "20 young plants. The competent authority may require a second submission of 20 plant for the second year of testing."

(iii) Conduct of Tests: Paragraph 1 to read: "To assess distinctness, it is essential to observe at least two satisfactory crops of fruit in two consecutive years."

(iv) Methods and Observations: The population standard to be 1 per cent with one off-type in 20 plants. Paragraphs 4 and 5 to be combined to read: "All observations on the stipule and the stolon should be made on one-year-old plants towards the end of the growing season." It was also clarified that the shadowgraphs mentioned in paragraph 7 would not constitute part of the description but only useful additional information.

(v) Table of Characteristics:

Characteristics

5 To have the states: "strongly concave (1), strongly concave to slightly concave (2), slightly concave (3), slightly concave to flat (4), flat (5), flat to slightly convex (6), slightly convex (7), slightly convex to strongly convex (8), strongly convex (9)"

8 To have an additional state "broader than long (1)" and to have the Notes from 1 to 4
9, 10, 18, 19, 31, 32 To have the Notes "1, 2, 3"

10 To have the states "serrate (Garriguette, Tenira, 1)" and crenate (Cambridge Favourite, Irvine, 2)"

20 To have the Notes "1, 2, 3, 4, 5"

21 To be deleted

22 To have the Notes "1, 2, 3, 4, 5," the states 2 and 4 to receive the addition "slightly"

24 To have the first state read: "reniform" and to have state 8 placed after state 5

26 To have the drawings improved by the expert from Germany

27 To have the states "medium, very strong" deleted and to have the Notes "1, 2, 3"

32 To read: "Fruit: insertion of calyx" with the states "in a basin level, above fruit"

34 To have the states "much smaller (1), slightly smaller (2), same size (3), slightly larger (4), much larger (5);" the expert from Germany to check the example varieties

36 To have the example variety "Holiday" for state 9

37 After this characteristic a new characteristic to be inserted reading: "Fruit: hollow center" with the states "absent or very weakly expressed (1), weakly expressed (2), strongly expressed (3)"

39, 40 The expert from Germany to indicate example varieties which have different states in the two characteristics in order to show that here is not a too strong correlation between the two characteristics

40 To have the asterisk deleted

41 To have the states 3 and 4 read: "fully remontant (non flowering runners) Brighton (3), day neutral (flowering runners), Florika (4)"

The expert from Spain stated that most of the example varieties indicated were not available or suitable in Spain. As in similar cases for other species, the competent authority would select example varieties which are more suitable for their testing purposes.

(vi) Literature: To have the following literature added:

- Guédès, M., 1981: No species names for strawberries ever published by A. N. Duchesne. Taxon 30: 299

- Guédès, M., 1984: Naming the dessert Strawberry (Rosaceae). Taxon 33: 724-5

- Hummer, K. E., 1993: *Fragaria* Catalogue (Strawberries). National Clonal Germplasm Repository, Corvallis, OR

- Hummer, K. E., 1993: *Fragaria* Catalogue (Strawberries). National Clonal Germplasm Repository, Corvallis, OR

- Rauschert, S., 1983: Zur Nomenklatur der Farn- und Blütenpflanzen aus dem Gebiet der DDR und BRD (VIII). Feddes Repert. 94: 289-302

- Roudeillac, P., 1994: 1st Inventory of Strawberry Collections (*Fragaria* spp.) maintained in the Research Stations in Western and Eastern Europe. 5.ed. CIREF, Prigonrieux, France

- Staudt, G., 1962: Taxonomic studies in the genus Fragaria Typification of Fragaria species known at the time of Linnaeus. Can J. Botany 40: 869-886.

Draft Test Guidelines for Cherry (Revision)

49. The Working Party noted document TG/35/4(proj.), proposals prepared by experts from Spain and comments received during the session and made only the following main changes in that document:

(i) General Decisions: The Working Party agreed to copy the following paragraph with their agreed amendments from the Test Guidelines for Apple: II(1) with the replacement of the names of the rootstock, II (2), III(3) and IV(1) but only with a 1 per cent population standard and no mutants, X(4.1, 4.2, 4.4) and the paragraph on GMO variety and on a photo to be added. It also agreed to always use the terms "ventral, dorsal, lateral" and not "front, back" when indicated from where the fruit and seed should be viewed.

(ii) Table of Characteristics:

Characteristics

20 To have the states "reniform, shaped, flat-round, round, oblong, correlate" and to have the example variety "Napoléon" deleted and to have the drawings amended by experts from France

21 To have the states "pointed (1), flat (2), depressed (3)"

28 To have the additional states "very low, very high" and the example variety "Montmorency (7), Meteor, Schattenmorelle (9)"

32 To read: "Fruit: abscission layer between stalk and fruit" with the states "absent (Burlat, Sunburst), present (Vittoria)," after this characteristic a new characteristic to be inserted reading: "Fruit: thickness of stalk" with the states "thin, medium thick" with example varieties to be indicated by experts from France

34 To receive drawings submitted during the session.

662

Draft Test Guidelines for Peach (Revision)

50. The Working Party noted document TG/53/4(proj.), proposals prepared by experts from Spain and comments received during the session and made only the following main changes in that document:

(i) General Decisions: The Working Party agreed to copy the following paragraph with their agreed amendments from the Test Guidelines for Apple: II(1) with the replacement of the names of the rootstocks, II(2), III(3), IV(1) with the changes as for the Test Guidelines for Cherry, X(4.1, 4.2 and 4.4) and the paragraphs on GMO varieties and a photo to be added.

(ii) Subject of these Guidelines: To have the spelling of the author "Batsch." in the Latin name corrected

(iii) Methods and Observations: To have the first sentence of paragraph 6 completed by the words "at the beginning of anther dehiscence"

(iv) Grouping of Varieties: To have characteristics 46 and 51 included as grouping characteristics.

(ii) Table of Characteristics:

Characteristics

1 To have the additional states "very small (Bonanza), very large (Champion)"

2 To have the bracketed words deleted

3. To receive drawings, to have the example variety "Weeping Flower Peach" deleted. To have the word "Nect." deleted in this and any other characteristics where it appears, the same to apply to "N." and to the word "Pavie"

5 To have the additional states "very short (Bonanza), very long"

8 To have the word "flower" added before "buds"

9 To have the word "general" from the states included in the characteristic

10 To have the states "non showy (1), showy (2)"

12 To have the states "narrow elliptic (1), broad elliptic (2), round (Springtime) (3)" and no drawings

14 To read: "Corolla: predominant color (inner side)" and to be placed before characteristic 12

17 To be deleted

24 To have the states "small, medium, large"

25 To read: "Leaf blade: shape in cross section"

27 To have the second state read: "approximately right angle"

29 To have the asterisk deleted and to read: "Leaf blade: color" with the states "green yellow, green, purplish read;" the expert from Japan to supply example varieties

34 To have the example variety "J. H. Hale" deleted

36 To have the states "prominently pointed (1), weakly pointed (2), flat (3), weakly depressed (4), strongly depressed (5)"

37 To read: "Fruit: symmetry (viewed from pistil end)"

41, 44, 45 To have the words "of skin" deleted

52, 53, 54 To have the states "absent or very weak (1), weakly expressed (2), strongly expressed (3)"

59 To be observed "in lateral view" and to have the states "oblate, round, elliptic, obovate" and to have the drawings amended

60 To read: "Stone: intensity of brown color;" after this characteristic a new characteristic to be added reading: "Stone: relief of surface" with the states "small pits (1), large pits (2), grooves (3), pits and grooves (4)"

(iv) Literature: To have two citations deleted (Breviglieri, N., 1950, Morettini, A., 1961)

(vii) Technical Questionnaire: To have the characteristic 5.7 (46) deleted.

Working Paper on Test Guidelines for Pear (Revision)

51. The Working Party noted documents TG/15/1, TG/15/1 Corr. and TWF/26/4 prepared by experts from Germany. It finally made the following main changes in document TWF/26/4:

(i) General Decisions: The Working Party agreed to copy the following paragraphs with their agreed amendments from the Test Guidelines for Apple: II(1) with replacement of the names of the rootstocks, II(2), III(3), IV(1) with the changes as for the Test Guidelines for Cherry and all paragraphs of X except for the characteristics. For the wording of the Technical Note discussions were based on those for Cherry.

(ii) Methods and Observations: To have the last part of paragraph 2 replaced by "10 plants or parts of 10 plants." Paragraph 3 to read: "Unless otherwise stated, all

observations on the tree and the one-year-old shoot should be made during winter on trees that have fruited at least once. The length of internodes should be observed in the middle of the shoots." In paragraph 5 the words "on at least 25 flowers" to be deleted and the whole paragraph to start with the words "Unless otherwise stated;" paragraphs 6 and 7 of TWF/26/4 to be replaced by paragraphs 8 and 9 from the draft Test Guidelines for Apple, with the exception of the deletion of the sentence on the terminal shoot in paragraph 8 and the replacement of the observation by "harvested at commercial harvest maturity and observed at peak maturity for consumption."

(iii) Table of Characteristics:

Characteristics

1 To have "Williams" replaced by "Williams Bon Chritien" throughout the Test Guidelines

2 To read: "One-year-old shoot: growth habit" and to be placed after characteristic 4

4 To have the bracketed content deleted and the first two states read: "fastigiate, upright"

5 To have the example variety "Luise Bonne" replaced by "Luise Bonne d'Avranche" in the whole Test Guidelines

6 To have the states "grey-brown (1), brown (2), orange-brown (3), brown-red (4), brown-purple (5), dark brown (6)"

8 To have the states "pointed (Williams, 1), slightly rounded (Passe Crassane, 2), rounded (Epine du Mas, 3)

9 To have the Notes "1, 2, 3" and the last state read: "strongly held out"

12 To read: "Young shoot: anthocyanin coloration of growing tip before liquification" and to have "Clapp's Favourite" as example variety for Note 1 and the example variety for state 7 corrected "Red Bartlett"

13 To read: young shoot: pubescence of upper part"

17 To have the states "very small, small, medium, large, very large" and to have the example variety "Doyenné du Comice" deleted

19 To have the states "acute (1), approximately right-angled (2), obtuse (3), flat (4), cordate (5)"

20 To have the states "acute (1), approximately right-angled (2), obtuse (3), rounded (4)"

21 To read: "Leaf blade: tip" with the states "absent, present" with another characteristic to read: "Leaf blade: length of tip" with the states "short, medium, long"

22 To have the word "indentation" replaced by "incisions" and to have the state "dentate" checked

23 To read: "Leaf blade: curvature in longitudinal section"

26 To have "Conférence" as additional example variety for state 7

27 To be placed after characteristic 30

29 To be placed before characteristic 27 and to read: "Flower: position of calyx in relation to corolla" with the states "adpressed, horizontal, recurved"

30 To have states inserted to be selected by the experts from Germany

31 To read: "Petal: length/width ratio" and to have "almost" replaced by "approximately"

33 To read: "Flower: position of style compared to stamen" with the states "below, same level, above"

36 To read: "Fruit: shape of lateral sides (in longitudinal section)" and to have the drawings amended

41, 42 To have the words "of skin (at eating maturity)" deleted

41 To have "Starkrimson" as example variety for the additional state "red (4)"

42 To read: "Fruit: amount of over color;" after this characteristic a new characteristic to be inserted reading: "Fruit: hue of over color" with the states to be proposed by the experts from Germany

49 To read: "Fruit: angle of basal part of stalk in relation to central axis" with the states "along axis (1), oblique (2), approximately right angle to axis (3)"

51 To be placed after characteristic 52

53 To have the example varieties "Coscia, Butirra Precoce Morettini" for Note 1

54 To have "breath" replaced by "width"

56 To have the states "rounded, elliptical, ovate"

57 To read: "Time of beginning of flowering"

58 To read: "Time of commercial harvest maturity"

59 To read: "Time of peak maturity for consumption"

To have the old characteristics 70, 71 and 72 reincluded, characteristic 72 to be split into two characteristics in case they are useful and actually used for distinctness and to have the observation methods checked.

(iv) Literature: To have the first citation deleted

(v) Technical Questionnaire: To be based on the Technical Questionnaire the draft Test Guidelines for Apple.

Future Program, Date and Place of Next Session

52. At the invitation of the expert from Israel, the Working Party agreed to hold its twenty-seventh session in Tel Aviv, Israel, from April 22 to 26 (noon), 1996. It was planned that the following items would be discussed during the forthcoming session:

(a) Short reports on new developments in member States in plant variety protection for fruit species (oral reports);

(b) Important decisions taken during the previous sessions of the Working Party, the Technical Committee and the Technical Working Party on Automation and Computer Programs (oral reports);

(c) Color observations and image analysis (Germany to collect information on research or use of image analyses);

(d) New methods, techniques and equipment in the examination of varieties (Netherlands to collect information on the use for screening reference varieties by the end of January 1996);

(e) Uniformity and stability in vegetatively propagated and self-pollinated varieties (United Kingdom to select apple mutation data (if available) for calculations with the TWC expert);

(f) UPOV Central Computerized Database;

(g) Relation between national listing and plant variety rights system (answers to a questionnaire to be collected by Israel);

(h) Discussions on working papers on Test Guidelines for:

- Apple Rootstocks (TG/14/5, TWF/26/11; the United Kingdom to prepare a new working paper by the end of January 1996).

- Citrus (Revision) (TG/83/3, TWF/23/6, TWF/24/3, Circular U 2234; South Africa to prepare a new working paper by the end of January 1996)

- European Plum (Revision) (TG/41/4, TWF/25/6; France to prepare a new working paper by December 15, 1995)

- Grape (TG/50/5, TWF/26/7 plus results of a Subgroup Meeting)

- Japanese Apricot (*Prunus mume*) (TWF/25/10, TWF/26/3; Japan to prepare a new working paper by December 15, 1995)

- Kiwifruit (TG/98/3, TWF/26/10; New Zealand to prepare a new working paper by the end of January 1996)

- Loquat (*Eriobotrya japonica*) (TWF/26/5; Japan to prepare a new working paper by December 15, 1995)

- Pear (TG/15/1 and Corr., TWF/26/4; Germany to prepare a new working paper by December 15, 1995)

- Pear Rootstocks (TWF/25/11, TWF/26/6 and TWF/26/9; Germany to prepare a new working paper by December 15, 1995)

- Prunus Rootstocks (TWF/24/4, TWF/25/4; France to prepare a new working paper by December 15, 1995)

- Walnut (TG/125/3, TWF/226/2; France to prepare a new working paper by December 15, 1995)

- Walnut Rootstocks (TWF/26/8; France to prepare a new working paper by December 15, 1995).

53. The Working Party agreed that comments on the existing drafts or working papers should be sent to the country which is preparing a new working paper, with a copy to the Chairman of the Working Party, before the end of October 1995. Only by using this procedure could it be assured that the new documents would have already taken into account most of the questions raised, and the discussions in the coming session would be restricted to the items where the opinions differed. Proposals for changes in the resulting new document should also be submitted in writing to the Office of UPOV to enable their distribution before the next session.

54. A Subgroup on Grape is planned to be held in Conneliano, Italy, possibly in the second half of January, 1996, to be organized by the expert from Italy. Experts from the OIV and IPGRI, as well as from the EU should also be invited to that meeting. Proposals for changes in the Test Guidelines for Grape should be sent to the Office of UPOV before November 15, 1995.

Visits

55. In the afternoon of September 12, 1995, the Working Party visited the National Fruit Collections at Brogdale, Faversham, where it received reports on the measuring of colors, on the measuring of apple shapes with image analysis and saw a demonstration of the database "Core store." It also visited the orchard with its national collections of mainly apples, pears and plums but also some other species as for example medlar (Mespilus).

56. In the afternoon of September 14, 1995, the Working Party visited Horticulture Research International at East Malling, where it received information on the breeding of apple, pear, raspberry and strawberry and visited the respective trial fields.

57. *The present report has been adopted by correspondence.*

[Eight Annexes follow]

ANNEX I

LIST OF PARTICIPANTS OF THE TWENTY-SIXTH SESSION OF THE TECHNICAL WORKING PARTY FOR FRUIT CROPS, CANTERBURY, UNITED KINGDOM, SEPTEMBER 11 TO 15, 1994

I. MEMBER STATES

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[Annex II follows]

ANNEX II

Report from France

U.P.O.V., Septembre 1995, U.K.

We always have lot of difficulties in identifying apples among which 70% of new varieties are more or less stable mutants. In peach species, identification problems are linked with the high number of new varieties and their rapid turnover. Consequently 25% of peach C.O.V. applications are withdrawn by the time the certificate is ready to be issued.

We also have important problems with the new rootstocks issued from interspecific hybrids which are often sterile. For this reason we think important to have in addition some physiological characters for distinction of varieties.

The analysis of biochemical and molecular markers in order to characterise new fruit material and in particular mutant, appears essential. The INRA Fruit Tree Stations in Angers and Bordeaux have invested for studies carried out with RFLP, RAPD and isoenzyme markers.

The Fruit Tree Research Station of INRA in Bordeaux is also in charge of the European *Prunus* Database under the authority of the ECP/GR *Prunus* group of IPGRI. This database involve 95 institutes holding *Prunus* collections from 23 countries in Europe and is to be available on Internet network by the beginning of 1996.

We also observed that breeders and editors are particularly reluctant to send their new material to country not admitted to U.P.O.V. (e.g. Morocco, Chilli, Argentina).

[Annex III follows]

ANNEX III

Oral report from Hungary

The plant varieties, including the fruit species can be protected in Hungary by patenting them since 1969. The Hungarian Patent Law was conformed to the International Convention for the Protection of Varieties of Plants in 1983 when Hungary became a member of the UPOV. Plant varieties can be patented by the National Office of Inventions. This office is an administrative authority, and the DUS tests are conducted by the National Institute for Agricultural Quality Control. From this year we have got a new Patent Law, but this law does not include the provisions of the new International Convention yet.

In Hungary the Office of Invention generally considers the results of DUS tests of a foreign variety, that is why we have to conduct the DUS tests with the varieties of Hungarian origin. In the last two years we tested one raspberry and six sweet chery varieties.

We conduct DUS tests not only for patenting varieties, but the varieties can entry on the National List on the basis of DUS tests and VCU (value for cultivation and use) trials, too. In Hungary - similarly to France - only the fruit varieties on the National List can be produced and marketed. That is why we conduct DUS tests mainly for national listing and only in a smaller quantity to protect the varieties. In the last years we had got many West European apple and small fruit variety applications for the National List.

If a variety is on the Natioanal List, the variety maintaining organization can collect a fee for using the variety from the nurseries. This system of variety using fees and the expensive patenting system result that only a few Hungarian fruit varieties are patented in Hungary.

Recognizing the fact that the EU-countries specialized on conducting DUS tests to certain species, Poland, the Czech Republic, Slovakia and Hungary divided the DUS tests to several agricultural and vegetable species. Hungary started to discuss with the countries I have mentioned, whether which countries could become the certain single testing centers for different fruit species, too.

[Annex IV follows]

Image analysis of apple fruit shape

Theofano Papageorgiou & Peter Dodd
Wye College,
University of London

Image analysis of apple fruit shape

- techniques currently in use for describing fruit shape:
  - length and breadth ratios
  - width at a series of different heights
    eg. pears (White,AG and Bailey,DG; 1994)
  - limacon shapes
    eg. peach (Cooke; 1970)
  - comparison with known shapes

Image analysis of apple fruit shape

Equipment:

- PC 486 33mhz c.p.u. VGA/SVGA monitor
- Image capture

  Digithurst MicroEye IC image capture card

  640 x 480 pixels

  16 colours in EGA or VGA modes
  256 colours in EVGA mode
  256 grey scale

  capture time ~ 4.5 seconds

- Camera

  Hitachi KP-C 500 all solid state colour camera

- Software

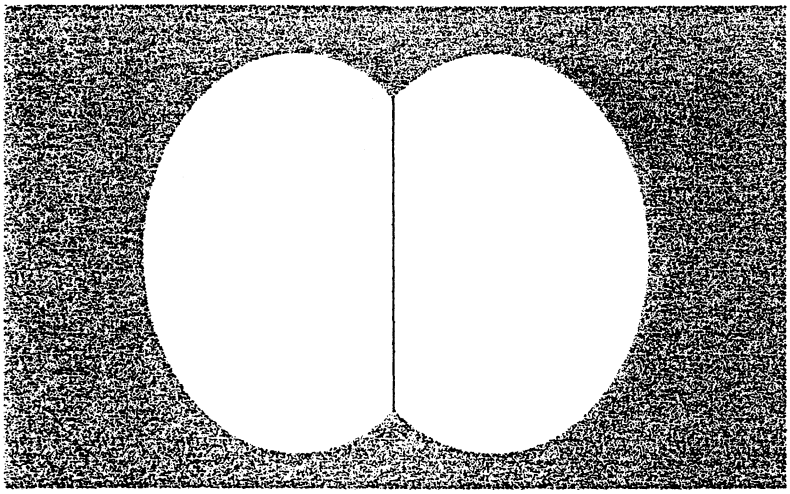
  Microsoft Windows 3.1

  Digithurst MicroEye IC software

  Pythagorus (Borland C++ for WindowsTM)

Image analysis of apple fruit shape

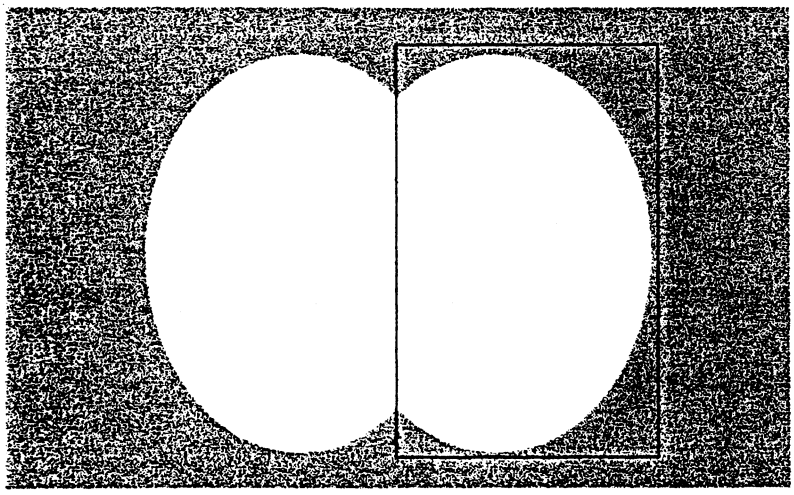
- **the process**
  - **fruit sectioned along longitudinal axis**



  - **cut surface can be photocopied for subsequent image capture**

    <u>or</u> the image can be captured straightaway

  - **scan taken of one half of the section**

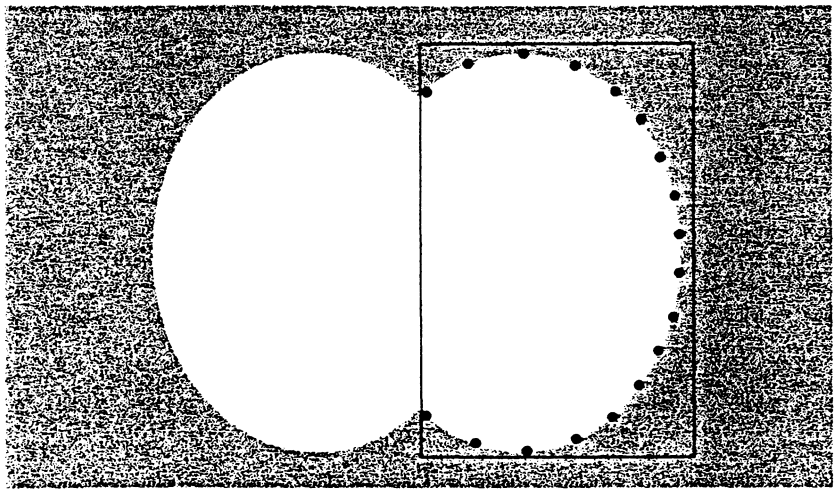


  - **image saved as a bitmap file**

APPSECTN.XLS

Image analysis of apple fruit shape

- **the process continued**
  - **image analysed using PYTHAGORAS**



shape is expressed as the co-ordinates of the points on the perimeter

the following are also calculated:

length of the perimeter

width:height ratio

area

- **the analysis of each image takes ~ 2 seconds**

SHAPEGRP.XLS

Image analysis of apple fruit shape

- **varieties for testing were selected from the following shape groups:**

1 flat

2 flat-round

3 short-round-conical

4 round

5 round conical

6 conical

7 long-conical

8 oblong-conical

9 oblong

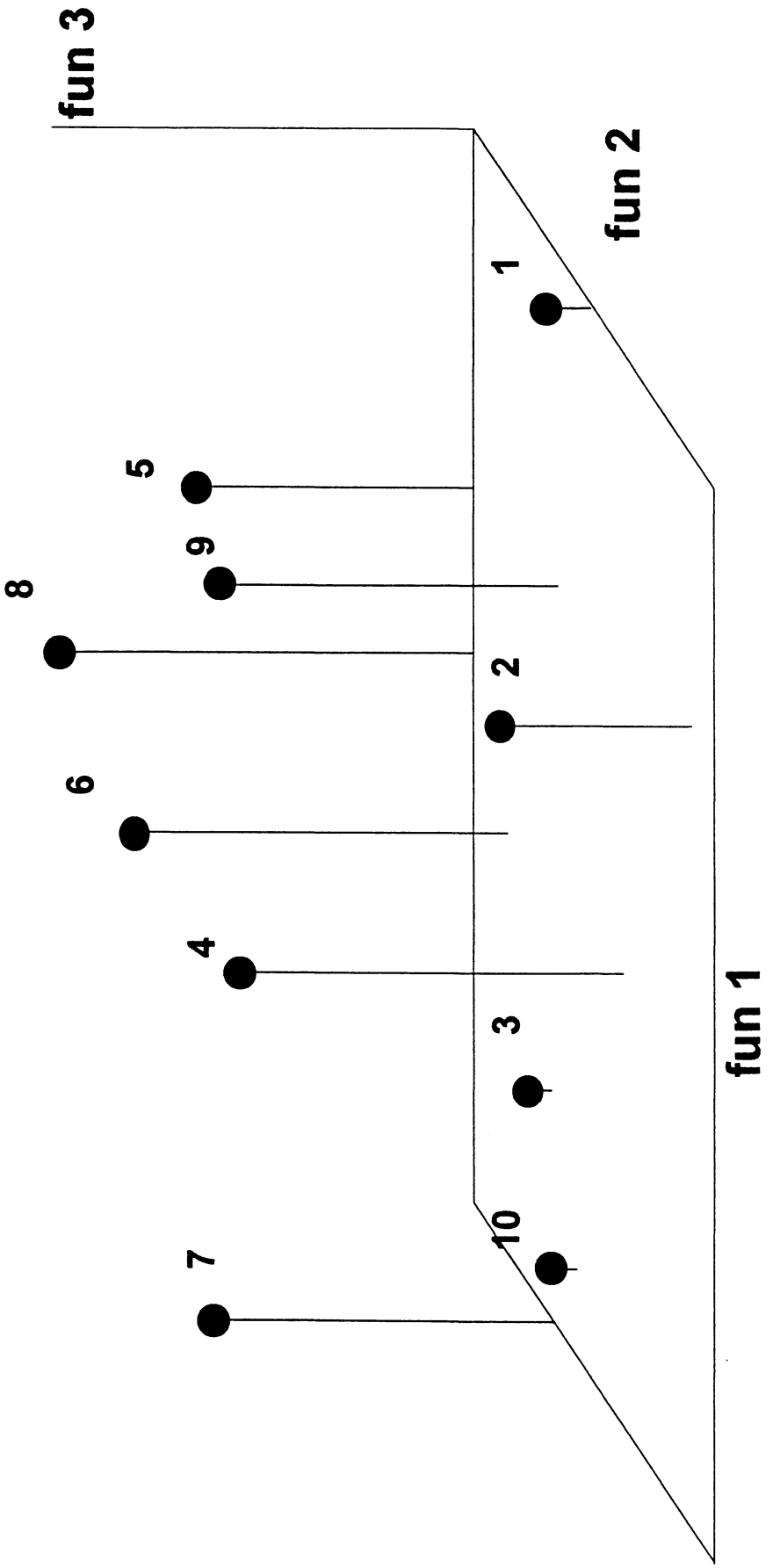
10 oblong-waisted

680

APPCANON.XLS

Image analysis of apple fruit shape

Discrimination of 10 apple shapes based on canonical functions



[Annex V follows]

ANNEX V

COLOUR MEASUREMENT OF CLONAL VARIETIES OF APPLES.

ALISON LEAN, WYE COLLEG
BROGDALE

When submitting clonal varieties of apples for Plant Variety Rights, the most frequent claim made by the breeders/applicants is of differences in fruit colour from the original variety. Colour is not easily assessed by eye. This is especially true of apple varieties where different amounts and shades of colour are present in different amounts over the surface of the fruit.

It is therefore useful for visual colour differences and similarities to be quantified. The use of tristimulus colour analysis to measure reflected colour has become widespread in fruit research, as this offers an objective measure of colour.

Colour measurements are taken using a Minolta CR-200 Chroma Meter. This has the advantage of being versatile and can be set to print and record in different colour spaces. (XYZ, red, green, blue; La*b*, lightness, red/green, yellow/blue; and LCH, lightness, colour, hue). These can be related to true colour. Colour measurements can be recorded from picked fruit in the laboratory or in the field with the use of batteries. Care has to be taken with recording fruit in the field as it is relatively easy to press the head of the Chroma Meter too firmly on to the fruit which causes bruising.

For most of the recording done to date, the La*b* colour spaces have been recorded for analysis. McGuire (1992) suggests that this is inappropriate as although L is correctly reported a* and b* are merely co-ordinates that indirectly reflect hue and chroma and are difficult to interpret in terms of actual colour. In our work with clonal varieties, the actual colour is not of prime importance as most of the varieties are very similar in colour. Using the La*b* notation system the colour is recorded in a set of numbers which can then be plotted or analysed to show the relationships between the colour of different fruit from the same clone and between different clones. These relationships have in every case confirmed visually assessed predictions.

METHOD

The Minolta CR-200 Chroma Meter is first calibrated on the standard white calibration plate CR-A43. The measuring head is placed flat against the fruit surface and the measurements taken. The multimeasure setting is used. This takes three measurements automatically, for better accuracy and prints the average of the three. For each fruit, three measurements are taken from different places on the part with the highest colour (assessed visually). This is usually on the cheeks and/or shoulder of the fruit. These three recordings are averaged to give one measurement per fruit for analysis. The background colour of the fruits can be measured in the same way. Additional characters which have been used in analysis, such as the amounts of high colour and background colour are estimated visually.

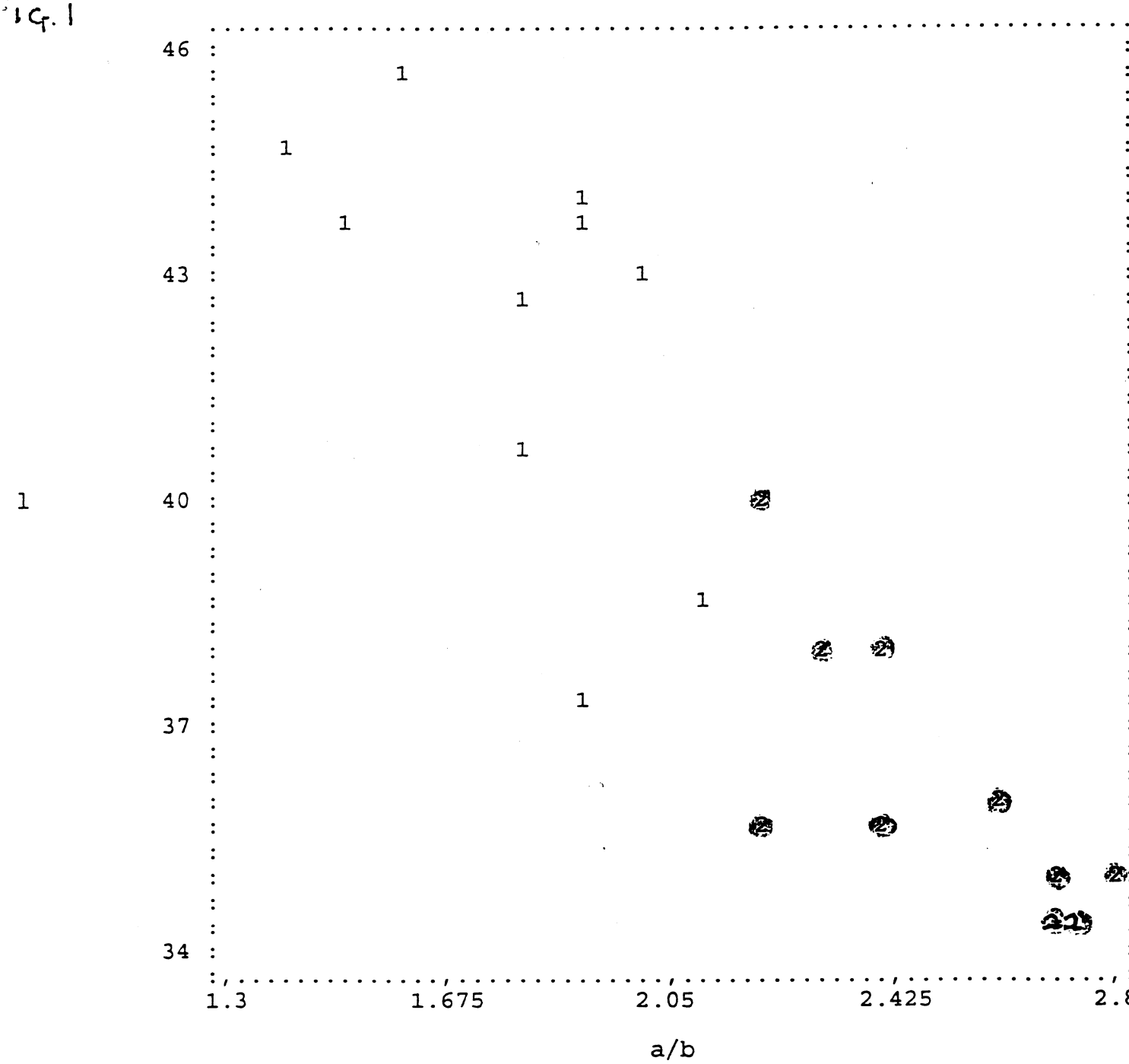
One drawback of the use of tristimulus colour analysis is that it is effectively limited to the assessment of block or uniformly coloured areas of the fruits. This is because the readings are the sum of the total pigment content (chromaticity) of the fruit skin. With striped or mottled coloured areas of the fruit the readings obtained are often extremely variable. (White, A.G. and Ngan, P.M. 1994) This confirms our results with recording non block coloured areas of the fruit. The recordings are often so variable that any statistical analysis is affected. For this reason the area of highest colour is used as in the clones in question this is usually block colour.

EXAMPLES

1. Comparing the colour of an original variety with the first clonal variety to be entered for PVR.
In pairs of varieties of this type, tested recently, there has been a clear visual difference in either the amount of colour or the intensity of colour. The fruit of the clonal variety is visibly darker red and a greater area of the fruit is coloured. In these cases scatter graphs of L plotted against a*, b* or a*/b* will show a clear separation of the two clones. t-tests can be used to compare the means of the two varieties for each of the three colour components. Fig 1
When a second clonal variety is submitted for testing with colour very similar to the first variety but clearly different to the original variety, it can be compared to the first clone in a similar way.

2. Some varieties, for example Jonagold and Elstar have large numbers of clones, some of which have passed (or failed) PVR while others are still being tested. For both varieties the clones can be grouped into those with lighter bright red fruit and those with darker coloured fruit. As the number of clones of the same original variety, named and submitted for PVR increases, the problems arise with trying to quantify the amount of

Fig. 1



The fruit colour of the clone (2) was visually darker and more consistant than the colour of the original variety (1). A greater surface area of the fruits of the clone was highly coloured

variation in colour within each clone and between different clones. There is often a large amount of variation in fruit colour between fruits from one clone and the average colour often appears to vary continuously within the clone and between the fruit of different clones. Visually there is no clear separation of clones by colour alone.

Multivariate statistics have been used as this makes it possible to incorporate information about the relationships of the variables. In the first example the La*b* measurements of seven Elstar clones were compared using Discriminant Analysis. In Discriminant Analysis the groups are known.

The means and standard deviations for each of the colour measurements are shown below.

CLONE	L	a*	b*
1	35.5 + 1.5	36.7 + 3.0	11.6 + 2.2
2	34.8 + 1.3	36.6 + 2.0	11.5 + 2.0
3	34.2 + 1.0	34.7 + 1.6	9.5 + 1.0
4	31.1 + 0.5	30.3 + 2.0	7.3 + 1.0
5	30.7 + 0.5	25.0 + 2.6	5.0 + 1.0
6	30.4 + 0.7	23.1 + 1.8	4.0 + 1.0
7	31.7 + 0.8	25.4 + 4.0	5.2 + 1.8

The a* measurements for varieties 1 and 7 show the greatest variation as expressed by larger standard deviations. This may in part be explained by differences in the ages of the trees. The trees in clones 1 and 7 were 4 years old, in their second year of fruiting, whereas the trees of the other clones were older (5 to 8 years). In this analysis 1 and 2 are two different accessions of the same clone.

Relationships between pairs of variables are shown by the table of Total-Sample Correlation Coefficients.

	L	a*	b*
L	1.0	0.9003	0.9265
a*	0.9003	1.0	0.9724
b*	0.9265	0.9724	1.0

In this example, the variables are all very highly correlated and are giving the same information; i.e. for these variables in these varieties a simple graph the values of two of the variables would show as much information as to the distribution of the varieties as an analysis using all three variables.

Discriminant Analysis was performed to show whether it is possible to separate the 7 known groups on the basis of the available data, in this case the L a* b* measurements making up the fruit skin colour. The table below gives information about the canonical discriminant functions.

Canonical Discriminant Analysis

Function	Eigenvalue	% Variance	Cumulative %	Canonical Correlation
1	5.135	78.31	78.31	0.914876
2	1.192	18.18	96.49	0.737449
3	0.230	3.51	100.00	0.432293

The 1st function accounted for 78.31 % of the total variability and function 2 for a further 18.18%. The first two functions have high correlation values showing a strong relationship exists between the varieties and the first two discriminant functions. Function 3 is relatively weak and is unlikely to add further to our understanding of the differences between groups.
The graph shows the relative positions of the varieties given by the first two canonical functions.

The varieties are separated by L and a* by the first canonical function.
The lighter, brighter, red skinned varieties are grouped to the right hand side and the darker red varieties to the left.

The a*/b* relationship is separating the varieties by the second canonical function, i.e. actual the red colour without the effect of how light and bright the colour appears.
The variation in red colour shown by the lighter red varieties is greater than that of the dark red varieties, as shown by the greater spread within each variety.
The analysis confirmed visual assessments of the similarities in fruit skin colour.

Discriminant Analysis has also been performed incorporating the colour measurements with the measurements of the morphological characters of the fruit. The plotted distribution of individuals is almost identical to the plot using colour measurements alone. i.e. for the clones in question, the colour characters are the discriminating variables. This may not be true for a different set of varieties. When the morphological characters are used in an analysis without the colour variables, there is very little grouping of individuals of the same clone showing that the variation in size etc is continuous within the group of clones and is not showing any identifiable pattern.

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ELSTAR CLONES

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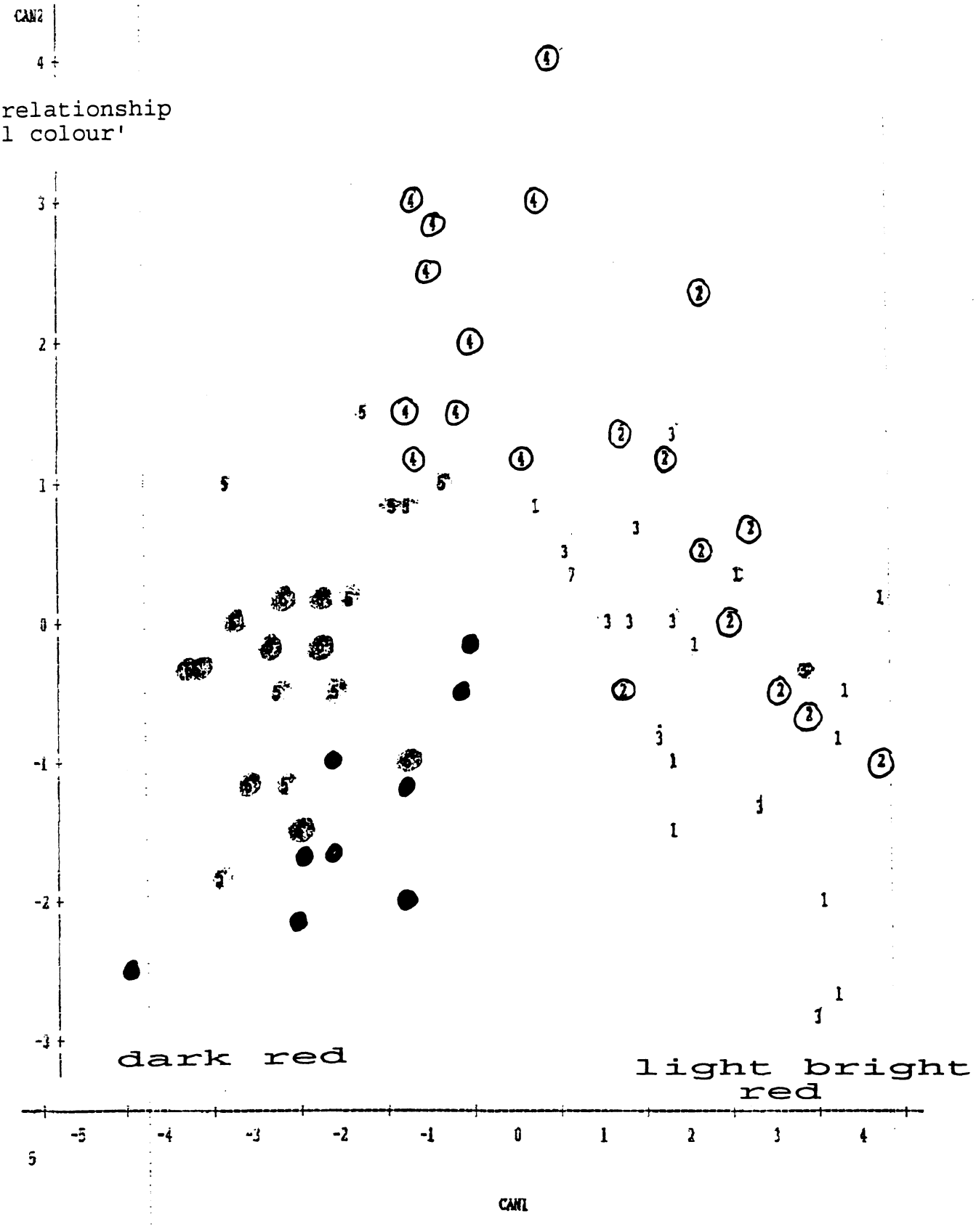
plot of canonical discriminant function

Plot of CAN2*CAN1. Symbol is value of TYPE.

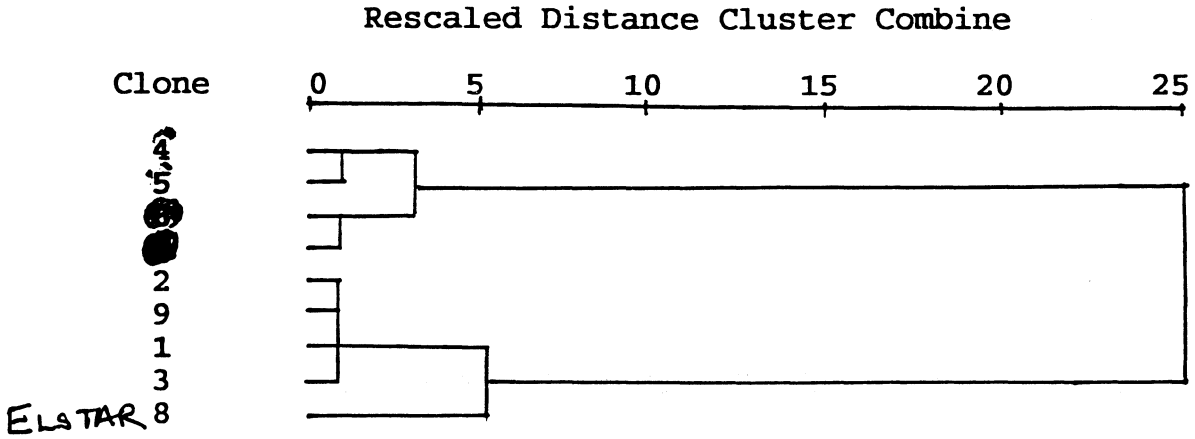


a*,b* relationship
'actual colour'

L, a* relationship
'lightness and red
element of colour'

CLUSTER ANALYSIS

Cluster analysis was performed using the means of the variables for seven clones. In cluster analysis the individuals are grouped according to similarities and differences in the data. The visual representation of steps in a hierarchical clustering solution is a dendrogram. The dendrogram, reading from left to right shows the clusters being combined and the values of distances at each step. The vertical axis lists the observations in a particular order. The order of the observations is useful in indicating which observations are sufficiently similar to be combined in the early steps. The horizontal axis shows the distances rescaled in the range 1 - 25. The largest distance corresponds to the value 25 and the smallest to 1. Large distances in the sequence indicate that at least two very different observations exist. Usually the major decision as to whether the cluster analysis has been successful should depend on whether the results make intuitive sense.
The results of the analysis are summarised below.



SOLUTION

GROUP	CLUSTER	CLONE
	1	4,5
A	2	6,7
B	3	3,2,9,1
	4	8

There are two main groups which correspond to the light bright red clones and the dark red clones. The clusters show the relationships of the different clones. There are four clusters. Clone 8 is original Elstar. 3,2,9,& 1 are the light bright red clones. The four clones making up the other group show less separation than do the light red clones from the original variety. Visually, the colour of all four clones is very similar, with clones 6 and 7 being slightly brighter due to a shiny skin finish. The cluster analysis using colorimeter measurements of the fruit skin, again confirmed relationships thought to exist by visual observation.

Discriminant and cluster analysis have been performed in the same way with the Jonagold clones. The situation with these clones is complicated by the numbers of varieties involved. (At least 16 using only the varieties with PVR and the varieties at present in test.) The results for colour analysis in Jonagold follow the same pattern as with the Elstars, in that the light coloured varieties separate from the dark coloured varieties but there is continuous variation in colour both between and within varieties. Both the Elstar and Jonagold clones show no difference in background colour. The amount of colour is related to the actual colour. Light red clones have greater amounts of background colour than do dark red clones.

Measurements of fruit skin colour in the problem clonal varieties have enabled us to quantify the character, and to examine it statistically. All results so far have confirmed visual assessments of similarities or differences between different clones, and the continuous nature of the variation in the character. The techniques could be used as confirmation of a visually assessed decision but at the present time we have no intension of basing decisions on the statistical results of colour measurement.

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[Annex VI follows]

ANNEX VI

Preliminary evaluation using Image Analysis to determine % Red Colour

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Introduction

Red colour is commercially important in apples. Slight variations in colour intensity are significant in the market place. A large number of colour enhanced sports of the NZ cultivar 'Braeburn' have been found. As of 30/6/95 2 'Braeburn' sports 'Hillwell' and 'Lochbuie' have plant Variety Rights (PVR) and another 8 'Braeburn' sports have provisional rights (NZPVRJ, 1995).

For new sports to obtain a PVR there have to be consistent differences in respect of one or more characters from existing cultivars. Colour is one possible discriminating variable. Many of these new 'Braeburn' sports claim to have higher % red colour or different intensity of red colour than standard 'Braeburn'. White et al 1991 reported that there was a positive relationship between the % red colour and intensity of colour in sports of 'Royal Gala'. In order to determine whether these new sports are distinct it is important to quantify % red colour differences.

The technique of image analysis is a useful tool in giving an objective quantitative measure of % red colour (White AW 1991).

Methods Materials

50 fruit were randomly sampled from 5 trees of 4 'Braeburn' sports ('Lochbuie','Hillwell', Sport A, Sport B) and standard 'Braeburn' ('Brillard') 2 days before commercial harvest. The trees, on MM106 rootstock, were planted in 1993 on a commercial orchard near Hastings. 40 fruit of uniform size were selected from the 50 fruit sample to minimise variability resulting from the curvature of the apple surface.

Fruit images were captured using an Ikegami model ICD-840P CCD camera linked to a Oculus-TCX True Colour frame grabber installed in an IBM compatible PC. Each apple was rotated 360° on a turntable as 256 single pixel width samples were taken along the longitudinal axis of the fruit. The video camera and turntable were synchronised so that the samples did not overlap. A near replica image of the fruit was constructed by stacking the samples side by side to build up a mosaic of the apple surface using VIPS5 software (Image Analysis Unit, 1992).

The red colour content was determined by counting the number of red pixels in the mosaic image and returning this as a percentage of the total mosaic area.

The overcolour pattern of each cultivar was described.

The data was analysed using SAS for PC. Means were compared by t-tests and differences declared significant if P>0.05.

Results

% Red colour

Differences in the % red colour were found between sports of 'Braeburn' in this trial (Table 1). 'Lochbuie', 'Hillwell' and Sport B all had an increased % red colour compared to standard 'Braeburn' 'Brillard'. There was no significant difference in % red colour however between 'Lochbuie' Sport B and 'Hillwell'. Sport A was not significantly different in % red colour to standard 'Braeburn' 'Brillard'.

Table 1 % Red colour of the skin 'Braeburn' sports

Sport	% Red Colour	
Lochbuie	66.4	a
Sport B	63.5	a
Hillwell	60.4	a
Sport A	38.3	b
Standard 'Braeburn' ('Brillard')	36.8	b

Mean separation by t-test (P=0.05)

Colour Pattern

'Lochbuie', Sport B, Sport A and 'Brillard' all had a striped over colour pattern. 'Hillwell' had a block overcolour with flecks.

Discussion

The 4 'Braeburn' sports tested have been selected by growers as distinctively more highly coloured than standard 'Braeburn'. 3 ('Lochbuie','Hillwell',Sport B) of the 4 sports were shown to have higher % red colour than standard 'Braeburn'. Sport A could not be distinguished from standard 'Braeburn'.

In order for Sport A to obtain a PVR it will have to be different from 'Brillard' in characters other than overcolour pattern and % red colour. Sport B could not be distinguished from 'Lochbuie' in characters of overcolour pattern and % red colour.

This present study with 'Braeburn' sports supports previous work using image analysis as a tool (White,1991) in quantifying % red colour in apple sports. Objectively quantifying % red colour in combination with other descriptions of the cultivars will help to differentiate sports.

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[Annex VII follows]

ANNEX VII

DIGITAL IMAGING , A USEFUL TECHNIQUE FOR ANALYSING FRUIT SHAPE IN PEARS

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Fruit shape in pears varies widely between cultivars. There are the calabash shapes of 'Abate Fetel' and 'Beurre Bosc', the ovate-pyriform shapes of 'Doyenne du Comice' and 'Ya Li', the conical shapes of 'Shinsei' and 'Hong Li' and the rounded forms of 'Nijisseiki' and 'Autumn Bergamot'.

Measurement of fruit shape has traditionally been based on comparisons of silhouettes of existing cultivars. Some descriptors incorporate ratios calculated from measurements of length and width (Tibault, Watkins and Smith, 1983).These ratios are intended to give an indication of the fruit shape irrespective of size. Thus a perfectly round fruit would have a height : width ratio of 1 and a relative height : max width of 0.5, whereas pyriform fruit would have figures for the same ratios of greater than 1 and less than 0.5 respectively.

Digital image analysis provides a quick and accurate means of measuring a range of parameters related to fruit shape. In the system we have developed, the fruit images are captured with an Ikegami model ICD-840P CCD video camera linked to a PCVISIONplus Frame Grabber installed in an IBM compatible Personal Computer (PC). The PC host computer performs the numeric computation and storage of data using the VIPS (Image Analysis Unit, 1992) image

processing software package.

Each fruit is placed on a spike, calyx end down, backlit to give good contrast and snapped with the video camera. The image is thresholded to produce a profile of the fruit against the background and the spike and stem are detected and removed from the image. The host computer then calculates the height, the maximum width, the height of maximum width from the base, and the widths at 10, 25, 50, 75 and 90% of the height from the base of the fruit (Fig 1.). The data is stored in an ASCII text file which can be imported into statistical package or spreadsheet for further analysis.

The measurements taken can be used for the calculation of ratios such as height : width, height of maximum width : maximum width and are also sufficient to allow a sketch of outline of the fruit to be reproduced graphically (Fig 2.).

Further developments planned include measurement of the concavity of the upper part of pyriform shaped fruit and the rate of taper from the point of maximum width to the base of the fruit.

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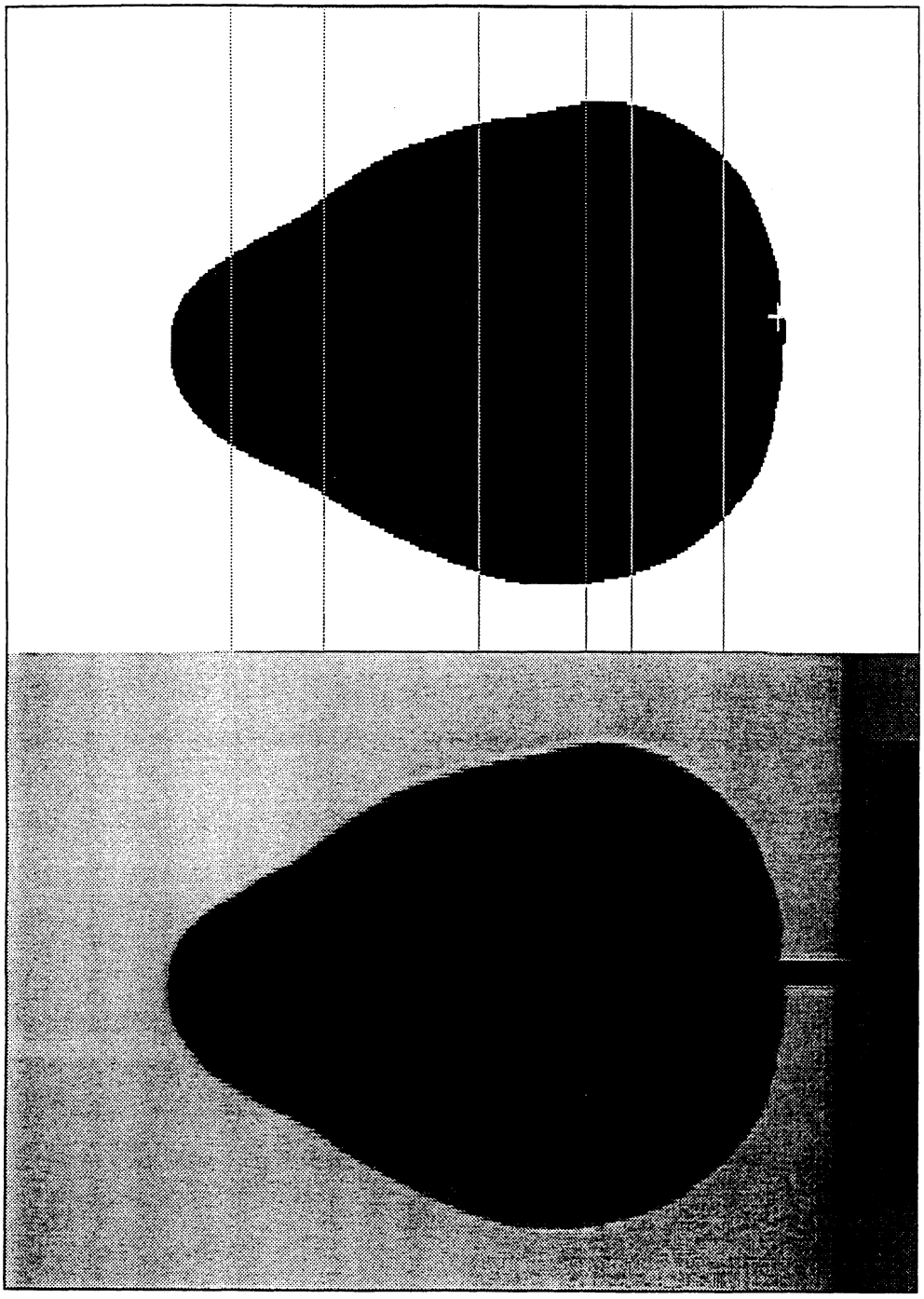
Anon. 1993. VIPS,Vision image processing system. Reference manual and users guide. Version 4.1. Massey University, Palmerston North.

Thibault, B., R Watkins and R.A. Smith.1983. Descriptor list for Pear. International Board for Plant Genetic Resources. IBPGR/82/57.



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ANNEX VIII

DIFFERENTIATING APPLE SPORTS BY POLLEN ULTRASTRUCTURE

The abstract from a thesis presented in partial fulfilment of the requirements for the degree of Master of Horticultural Science at Massey University, 1995.

Alastair Currie

Supervisors
Dr. G. S. Lawes, Dr. D. Noiton, and Dr. D. Bailey

ABSTRACT

Cultivars are plants that form distinct, uniform and stable phenotypes. New cultivars can be protected by Plant Variety Rights (PVR) which allow the owner exclusive rights to the propagation and sale of the plant material. Current PVR identification methods for apple cultivars require detailed records of tree, flower and fruit characteristics to differentiate the new cultivars from known cultivars. This method is slow, expensive and unable to cope with the increasing numbers of sports. Biochemical identification methods such as isozymes, restriction fragment length polymerisation (RFLP), random amplified polymorphism DNAs (RAPD), and minisatellite probes, can quickly and objectively differentiate cultivars, but cannot differentiate apple sports. Previous research suggested that pollen ultrastructure could be an alternative method for plant identification. This thesis is concerned with the development of a technique to differentiate apple sports using pollen exine patterns.

Scanning electron microscopy was used to capture images of the apple pollen grain and the exine surface. A digital image analysis algorithm was developed to extract quantitative data from the pollen grain dimensions and pore characteristics, and a Fast Fourier transform extracted quantitative data from the ridge patterns. Statistical methods were applied to the data to differentiate the sports.

Pollen harvested from apple flowers in the spring were wider than pollen harvested from flowers forced out of season under artificial conditions. Significant differences between trees were found for pollen grain length:width ratio, percent pore coverage, pore area and pore length but further research is required. However, apple cultivars types 'Red Delicious' and 'Gala' were successfully differentiated by pore and pollen grain variables, and 'Aversang' and 'Ultrared' sports of 'Red Delicious', and 'Splenola' and 'Galalea' sports of 'Gala' were successfully differentiated by exine ridge patterns and pollen grain measurements.

Differentiation of apple sports by pollen requires further development but may be one of the only quick, objective identification methods that can differentiate sports. Sport differentiation would greatly aid PVR establishment and enforcement.

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