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## INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

TECHNICAL WORKING PARTY  
FOR  
AGRICULTURAL CROPS

Twenty-third Session

Seville, Spain, May 17 to 20, 1994

## REPORT

adopted by the Technical Working Party  
for Agricultural Crops

**Opening of the Session**

1. The twenty-third session of the Technical Working Party for Agricultural Crops (hereinafter referred to as "the Working Party") was held in Seville, Spain, from May 17 to 19, 1994. The list of participants is reproduced as Annex I to this report.
2. Mr. Ricardo López de Haro y Wood welcomed the participants to Seville. The session was opened by the Chairman, Mr. Huib Ghijsen (Netherlands).

**Adoption of the Agenda**

3. The Working Party adopted the agenda of its twenty-third session as reproduced in document TWA/23/1 Rev.

**Report on the Twenty-second Session of the Working Party**

4. The Working Party recalled the report on its last session as reproduced in document TWA/22/17 and the fact that the Technical Committee had not yet noted the document.

**FINAL DISCUSSION ON DRAFT TEST GUIDELINES****Draft Test Guidelines for Wheat (Revision)**

5. The Working Party noted the draft Test Guidelines for Wheat as reproduced in document TG/3/9(proj.) and comments reproduced in document TWA/23/8. It finally agreed to amend Note 4 of characteristic 28 to read: "band 7 and, in the presence of bands 5 and 10 of characteristic 29, bands 7 and 9" and to amend in the explanations the last Note on page 31 to read: "Note: Certain bands (e.g. bands 9 and 10) have similar molecular weights. This leads to the fact that, in the presence of bands 5 and 10 of characteristic 29, two states of expression of characteristic 28, band 7 (Note 4) and bands 7 and 9 (Note 3), cannot be differentiated from one another. Therefore, in the presence of bands 5 and 10 of characteristic 29, Note 4 of characteristic 28 can be either band 7 or bands 7 and 9 (same as Note 3). Other bands having similar molecular weights can be differentiated from one another by their known association with other bands. For characteristic 28, band 13 is always associated with band 16 and band 14 with band 15 while band 40 remains alone."

6. The Working Party confirmed, at the request of breeders present in the session, that the inclusion of electrophoretic characteristics in the draft Test Guidelines for Wheat did not mean that from now on they would be used as routine characteristics. They were included without an asterisk and most countries intended to use them only as a last resort if a new variety could otherwise not be distinguished from an existing variety. They would only be used with the agreement of the applicant. If they were used, however, the candidate variety would have to be uniform in those characteristics as well as the variety from which it otherwise could not be distinguished. The characteristic would then also be used in the further multiplication to check whether the breeder had kept his variety uniform.

**Draft Test Guidelines for Barley (Revision)**

7. The Working Party noted the draft Test Guidelines for Barley as reproduced in document TG/19/8(proj.) and approved it without any changes. Breeders present at the session raised the same concern as over wheat, and reference was made to the answers given in that context (see paragraph 6 above).

**Draft Test Guidelines for Maize (Revision)**

8. The Working Party noted the draft Test Guidelines for Maize as reproduced in document TG/2/4(proj.) and the report of the Subgroup and comments reproduced in documents TWA/23/3 and TWA/23/4, as well as further information on the outcome of the Subgroup meeting held on May 16, 1994. It agreed to amend document TWA/23/4 by adding at the end of paragraph 11 the words: "especially for Mdh3 and Mdh5," replacing in the last line of paragraph 12 the word "separate" by "interpret" and rewording paragraph 14 as follows: "The Subgroup noted that the above presentation would still leave a problem for some genes (Mdh, Acp, Pgm, Pgd, Idh), in which interactions between different products of the genes or overlapping of bands would occur. While in homozygous inbred lines the known interactions could help separate each characteristic, for hybrids a separate interpretation of the single genes was impossible. The Subgroup therefore agreed to have six separate Mdh characteristics for inbred lines but combined characteristics of those Mdh genes for hybrids."

9. The results of the discussions in the Subgroup on May 16, 1994 (for the list of participants see Annex II to this report), and of the discussions on Maize at the session of the Working Party are reproduced in document TWA/23/15. The Working Party asked for that document to be prepared and presented to ASSINSEL the week following the session, in order that comments on the draft might be presented in time for those Test Guidelines to be also presented to the Technical Committee for final adoption at its November session. The main concerns in the discussions were the sample size, the indication of tolerances for off-types and whether to follow the new proposal to indicate the population standard and acceptance probability or to indicate the maximum number of off-types acceptable for a given sample size, as had been done in the draft Test Guidelines for Wheat, Barley and Oats. For the latter draft Test Guidelines it was considered that more knowledge was necessary and that it was too early to change the presentation. The final decision (to indicate the population standard) is reproduced in paragraphs 5 and 6 of document TWA/23/15. Another concern was whether or not to separate the characteristics on electrophoresis for inbred lines and hybrids. It was finally decided that they would not be separated, with the exception of Pgm1 + Pgm2 and Acpl for which separate characteristics were foreseen for inbred lines and for hybrids (see document TWA/23/15, characteristics 43 and 45).

#### Survey on the Use of Electrophoresis in Potato

10. The Working Party noted document TWA/23/9 containing a survey prepared by experts from Germany on the use of electrophoresis in potatoes. It noted that electrophoretic characteristics had good discriminative power but it was not possible to use them to discriminate between all varieties. In the discussions different views were expressed concerning the inclusion of electrophoresis in the draft Test Guidelines for Potato. Some experts expressed the opinion that, as potatoes were vegetatively propagated, the maintenance of the reference collection was an expensive exercise. The systematic observation of electrophoretic characteristics could permit the building up of a data base which would facilitate the selection of similar varieties to be grown, thereby reducing the costs for field tests and the maintenance of too many varieties. That, however, would require clear description and interpretation of the method and good calibration of the gels. Other experts warned against going too far. A system such as the above would in fact reverse the situation and almost make the morphological characteristics into "last resort" characteristics. There were sufficient morphological characteristics for distinction. In most cases mutations could not be distinguished. As for cereals, electrophoresis should be used only as a last resort. Breeders present at the session declared themselves in favor of the use of electrophoresis. The Working Party finally agreed to set up a Subgroup on Potato which would meet in Hanover, Germany, in November 1994 to discuss the possible inclusion of electrophoretic characteristics in the Test Guidelines for Potato.

11. During the session the possibility of using electrophoresis in other agricultural species was also discussed. In order to have a better basis for discussions during the next session, the Working Party agreed that various experts would prepare, before the end of the year, documents for several species as follows:

Poa pratensis:	Mr. Ghijsen (NL)
Ryegrass	: Dr. Camlin (GB)
Timothy	: Mr. Guiard (FR).

### Discussion on New Technologies

12. The Working Party noted the summary report given by Mr. Guiard, Chairman of the BMT, on the results of the last session of that Working Group held in Versailles, France, from March 21 to 23, 1994. The full report on the session will be reproduced in document BMT/2/9 Prov. It needed to be made clear whether the new methods would be used to establish essential derivation or DUS. For DUS purposes, it was reproducibility between laboratories and countries that required special attention, as well as knowledge of the genetic background and of the correlation with phenotypic expressions or the link between genotype and phenotype. A large number of papers on different species would be prepared for the next session (in Wageningen, Netherlands, from September 19 to 21, 1995) in order to afford broader knowledge of the situation regarding those species.

### Cooperation With Breeders in the Testing of Varieties

13. The Working Party noted document TWA/23/7, containing a summary of the survey on the involvement of the applicant or breeder in the examination of a variety based on trials carried out by or on behalf of the breeder, according to the conditions laid down in Annex II to document CAJ/32/10-TC/29/9, approved by the Council in 1993. The Working Party noted that some countries had had difficulty in understanding certain questions. It asked for comments to be sent to the Office of UPOV in order to improve the questionnaire and circulate it once more. The Working Party encouraged all countries to answer the questionnaire so that there might be a better understanding of how testing was done in the various member States. Other Working Parties should also collect similar information and the Technical Committee should be involved as well. It would be of special interest to know why countries had chosen certain testing systems for certain species.

### UPOV Central Computerized Data Base

14. The Working Party noted the history of the discussions concerning a possible UPOV central computerized data base as set forth in document CAJ/32/2-TC/29/2 and Circulars U 2047 and U 2067 and that the Council, during its session in October 1993, had approved the preparation of a prototype for a UPOV data base. It also noted the preparation of a UPOV format for the transmission in electronic form to a UPOV central computerized data base on CD-ROM of bibliographic data regarding plant varieties as reproduced in document TWC/12/8. That format will now be given to a firm to develop a prototype on the basis of data to be supplied in that format by the Offices participating in the ad hoc working group. The Working Party welcomed the progress made and hoped to receive the first results of the testing of the prototype as well as information on the steps to be taken on the basis of those results at its next session. It expressed the hope that most member States would finally participate in the data base so that all varieties might be covered.

### Statistical methods

15. Mr. Guiard (France) reported that his enquiry about the handling of off-types in the adopted Test Guidelines for agricultural crops had revealed that in most Test Guidelines the same population standard had been applied. He had therefore put more emphasis on the criteria for the selection of the right population standard. In discussions with the national statistics experts it had become clear that the population standard had to be chosen according to

the objectives, the control standards, the reproductive biology, the seed generation, etc. It was not the experimental layout which was decisive for the population standard (not even indirectly, because, taking into account the effort necessary (number of plants to be observed), it was impossible to fix the population at the (low) level aimed at in the beginning). The test had to be made by looking at all the characteristics and not characteristic by characteristic. The statistics presented so far did not allow account to be taken of the fact that it could be more or less difficult to recognize whether or not a given plant was an off-type. Nor could they take into account that there were more chances of finding off-types when many characteristics were observed rather than just one. Mr. Guiard then continued to explain in detail the connection between the different parameters and their effect on the alpha and beta risks, on the basis of tables reproduced in Annex III to this report. The efficiency curve for homogeneity on page 1 of Annex III was discussed at length.

16. The Working Party agreed that a high beta risk (risk of wrongly accepting a heterogeneous variety as uniform) was a risk not only for the user but also, and possibly even to a larger extent, for the breeder. Another breeder could make selections in that variety. It was also a risk for the authority, which might take bad decisions, and for the plant variety protection system in that it made distinction more difficult. Breeders would have no interest in presenting heterogeneous varieties, however.

17. The Working Party concluded that the decision on the right population standard was a matter for the technical expert; statisticians could only give guidance as to the criteria for selection. However, it still had difficulty in fully understanding the criteria for the selection of the right population standard and the right acceptance probability, which would lead to a number of off-types being considered the right ones based on past experience.

18. The main question was whether the population had to remain the same, regardless of the type of trial, with only the acceptance probability changing ( $\alpha_1$  for ear-rows and  $\alpha_2$  for drilled plots) in order to reach the number of off-types accepted at present (e.g. 3 in 100 ear-rows, 5 in 2,000 for drilled plants) of wheat, or should the acceptance probability be kept the same for both trials and the population standard adjusted ( $P_1$  for ear-rows and  $P_2$  for drilled plots) depending on whether ear-rows or drilled plots were being considered. Other experts felt that, as characteristics in drilled plots were observed together, while in ear-rows they were observed individually (e.g. drilled plots with the naked eye compared to ear-rows with a magnifying glass), a different population standard was applicable for the observation of individual characteristics as opposed to the observation of several characteristics together. Others considered that different population standards were justified because of different generations looked at, and still others considered that the ears sent in for ear-rows in wheat might have been more carefully selected by the applicant than seed and would therefore require different treatment.

19. The whole question would thus require further study and discussions with statistics experts. This should not, however, delay the adoption of the cereal Test Guidelines, which should be presented to the Technical Committee for adoption with their present wording with respect to uniformity. These discussions on the reasons for different treatment for ear-rows and drilled plots in cereals, together with good practical experience, could lead to a better understanding and a global answer, which could also be helpful for subsequent application to crops where less experience was available. The main problem was to find the right questions to be put to the statisticians in order to secure help from them.

20. Mr. Ghijsen (Netherlands) finally offered to prepare a document on the question of the selection of the right population standard and the acceptance probability for ear-rows and drilled plots for the next session of the Working Party.

21. The majority of the Working Party clarified that the terms "a single observation of a group of plants or parts of plants" and "a number of individual ear-rows, plants or parts of plants" in Chapter IV of the Test Guidelines applied to uniformity only, and should not be confused with the indication of the abbreviations "VG" or "VS" in the Table of Characteristics, which had the same meaning but applied to distinctness only and said nothing on the testing of uniformity.

22. The Working Party confirmed its proposal to clarify the range of application of documents TC/30/4 and TWC/11/16 and to combine them in a single document of which document TWC/11/16 would form Part I, applicable to vegetatively propagated and self-fertilized crops, while document TC/30/4 would be Part II, applicable to cross-fertilized crops. As the wording of document TC/30/4 was not yet sufficiently simple for easy understanding, Dr. Camlin (United Kingdom) would contact the authors (Dr. Weatherup and Dr. Talbot, United Kingdom) and cooperate with them in producing an amended, simplified version. At the same time, the document should also state, in a similar way to document TWC/11/16, the necessary alpha-risk and beta-risk figures and advise on the risks taken if applied to other crops. It should, furthermore, make reference to the Long-Term LSD method and its use in cases of less than 20 varieties and less than 12 degrees of freedom.

23. Mr. Bar-Tel (Israel) gave a short report on the outcome of the discussions of the last session of the TWC. The full report would be reproduced in document TWC/12/11 Prov. The next session of the TWC was scheduled to be held in Slupia Wielka, Poland, from June 7 to 9, 1995.

#### **Testing of Resistance to Diseases**

24. The Working Party noted document TWA/23/10 containing a summary of discussions in UPOV on resistance to diseases in DUS testing. It also noted the following three main questions: (i) whether to use only cases of clear absence or presence, (ii) whether to use only clear resistance or also tolerance and (iii) whether to include them in the Test Guidelines but without an asterisk. The Working Party repeated that in agricultural species resistance was, in principle, used only as a last resort. The Working Party was, however, aware of the fact that the situation was different in other groups of species, and that for vegetable species resistance characteristics were in many cases used as grouping characteristics. The decision whether to use resistance characteristics for distinctness would therefore depend very much on the species concerned and the genetic bases.

25. The Working Party confirmed the rule that an asterisk could only be given to a characteristic if all member States agreed. There was no special rule for resistance, and so that rule should apply also to resistance characteristics. The decision would have to be taken species by species and characteristic by characteristic.

26. In many cases resistance was not a black and white situation, and different degrees of resistance existed. This fact as such was not a problem for the acceptance of the characteristic as long as there was a good description of each state of expression.

27. The question whether the term "tolerance" and tolerance characteristics were acceptable in UPOV Test Guidelines occupied a large part of the discussions. The Working Party agreed to the definition of the terms given by breeders and reproduced in Annex IV to this report. It noted that in many cases tolerance characteristics would not be acceptable for distinctness purposes. However, the mere fact of its being a tolerance characteristic would not always preclude its use for distinctness. As with any other characteristic, if all normal requirements were fulfilled, a tolerance characteristic could also be included in the UPOV Test Guidelines.

28. The Working Party asked the Office of UPOV to distribute document TWA/23/10 also to the TWV.

#### **FINAL DISCUSSION ON DRAFT TEST GUIDELINES**

##### **Draft Test Guidelines for Oats (Revision)**

29. The Working Party noted the draft Test Guidelines for Oats as reproduced in document TG/20/8(proj.), and approved them without any further changes.

##### **Draft Test Guidelines for Fodder Beet**

30. The Working Party noted the draft Test Guidelines for Fodder Beet as reproduced in document TG/150/1(proj.) and approved them without further changes during the session. The expert from France would, however, check whether further literature could be cited in the document.

#### **DISCUSSION ON WORKING PAPERS ON TEST GUIDELINES**

##### **Working Paper on Test Guidelines for Rape (Revision)**

31. The Working Party noted documents TG/36/3, TWA/22/4 and TWA/23/5 and made the following main changes to document TWA/23/5:

(i) Material Required: To increase the minimum seed per component to be supplied from 50g to 100g (for two sowings).

(ii) Table of Characteristics: To delete the question-marks in characteristics 20 and 21.

(iii) Technical Questionnaire: To mention the following subgroups in paragraph 4.1 under (i):

- "- male sterile line
- maintainer
- restorer"

and the following subgroups under (iii):

- "- male sterile hybrid
- restorer hybrid."

32. The Working Party noted that for characteristic 11 (Time of flowering) no agreement had yet been reached on whether to observe it on individual plants or on the plot as a whole. In addition, different opinions existed on the use of plant rows and on whether male sterility was acceptable as a distinguishing characteristic. This would make it difficult to take over test results obtained in other States. Further efforts would therefore be necessary to



obtain more harmonization, so the Subgroup on Rape was asked to meet again in Versailles, France, in (the beginning of) 1995. In the meantime, more example varieties would be selected during the present session, the tolerance for hybrids would be studied further and possibly agreed upon by correspondence, and the question of male sterility in hybrids would be investigated further.

**Working Paper on Test Guidelines for Flax (Revision)**

33. The Working Party noted documents TG/57/3, TWA/20/5 and TWA/23/11 and made the following main changes to document TWA/20/5:

(i) Material Required: In paragraph 1, to use the new wording and to change the number of unthreshed plants from 150 to 100.

(ii) Conduct of Tests: To change, in paragraph 3, the number of plants from "about 2,000" to "at least 1,000" and in the following sentence to delete the words "at least" before the number 80.

(iii) Grouping of Varieties: To have the grouping characteristics:

"(i) Petal: color (characteristic 5), but with the states white, blue, pink, violet

(ii) Boll: ciliation of false septa (characteristic 11)"

and to also use the following further grouping as included under paragraph 7.2 of the Technical Questionnaire: "Use" with the groups "fiber, oil, both (fiber and oil)."

(iv) Table of Characteristics:

To include a new column, as in the Test Guidelines for cereals, for the indication of the way in which the characteristic should be observed:

M: actual measurement

VG: visual assessment by a single observation on a group of plants or parts of plants

VS: visual assessment by observations on a number of individual panicle-rows, plants or plant parts.

The following characteristics to receive the following indications:

M: 2, 12

VG: 3, 5, 5a, 10, 13, 14

VS: 1, 2, 4, 6, 7, 8, 9, 11

**Characteristics**

1a To be deleted and included in the Technical Questionnaire

2 To receive the addition "(when fully developed)"

5 To read: "Petal: color of corolla (when fully developed)" and to have the example variety for Note 4 reading: "Olinetta (0)"

5a To be placed before characteristic 5 and to be observed "just before opening of flower"

7 To read: "Stamen: color of distal part (at opening of flower)"

- 8,9 To be observed "(as for 7)"
- 9 To have the additional state "yellow" inserted before "blue"
- 13 To have the states "yellow (Hella (0)), green, light brown (Ocean (0)), medium brown (Antares (0)), dark brown (Viking (F), Mikael (0))"
- 14 To be deleted
- 15 To have the states with even Notes deleted, and those with Notes 1 and 9 added

(v) Literature: To have the literature from document TWA/23/11, page 4, included.

(vi) Technical Questionnaire: To have paragraph 5.1 deleted and under paragraph 7.2 the following added:

- "(i) Use
- fiber
  - oil
  - both (fiber and oil)
- (ii) Time of sowing
- winter
  - spring"

**Working Paper on Test Guidelines for Soya Bean (Revision)**

34. The Working Party noted documents TG/80/3, TWA/22/6, TWA/23/2, TWA/22/17, paragraph 44, and TWA/23/2 Rev. and made the following main changes to document TWA/23/ Rev.2:

(i) Table of Characteristics:

Characteristics

- 2 The expert from France to supply the method before the end of the year
- 4.3 The expert from France to prepare a drawing
- 7.2 To transfer the explanations of the states to the chapter "Explanations"
- 7.3 To have the state "brown" split into the three states "light brown(4), medium brown(5), dark brown(6)"
- 7.4 The experts from France to check whether the characteristic could be replaced by electrophoretic characteristics
- 8 To read: "Plant: time of beginning of flowering (one flower open on 10% of plants)"; the characteristic "Plant: time of maturity" to be reincluded in the Table
- 9 To have the states "absent, present"
- 10 For the time being not to be included, pending the outcome of the discussions in the BMT

(ii) Electrophoretic Characteristics: The Working Party confirmed its decision to include electrophoretic characteristics in the Test Guidelines for Soya Bean. The experts from France would prepare a proposal for those characteristics before October 1994.

(iii) Example Varieties: The experts from the United States of America and France would exchange example varieties in order to produce an agreed list for inclusion in the Test Guidelines.

#### Working Paper on Test Guidelines for Subterranean Clover

35. In the absence of an expert from Australia, the Working Party decided to postpone the discussion of document TWA/23/6 until its next session. It asked all experts to send their comments on the document to Australia for analysis and compilation.

#### Working Paper on Test Guidelines for Rice (Revision)

36. The Working Party noted documents TG/16/4 and TWA/23/12 Rev. and made the following main changes in the Table of Characteristics of document TWA/23/12 Rev.:

##### Characteristics

- 5 To read: "Flag leaf: attitude of blade" with the states "erect, semierect, horizontal, reflexed"
- 13 To be split into three characteristics:
- 13(a) "Stem: anthocyanin coloration of nodes" with the states "absent, present"
- 13(b) "Stem: intensity of anthocyanin coloration of nodes" with the states "weak, medium, strong"
- 13(c) "Stem: anthocyanin coloration of internodes" with the states "absent, present"
- 15 To be kept for the time being but further checked
- 16,20,22,30 To be kept in their present form in the Test Guidelines
- 17 To be deleted
- 20 The two additional characteristics after characteristic 20 to read:
- 20(a) "Panicle: compactness" with the states "open(3), intermediate(5), compact(7)"
- 20(b) "Panicle: exertion of base" with the states "partly exerted(3), exerted(5), well exerted(7)"
- 27 The additional characteristic after characteristic 27 to read:
- 27(a) "Decorticated grain: shape in lateral view" with the states "round(1), semi-round(2), half-spindle-shaped(3), spindle-shaped(4), very spindle-shaped(5)"; no example varieties would be indicated: the ratio for each state of expression would be mentioned in the explanations instead

30 To be checked by the Spanish experts; the additional characteristic after characteristic 30 to read:

30(a) "Aroma" with the states "absent or very weak(1), weak(2), strong(3)"; the experts from Spain to supply the method for this characteristic

37. The experts from Spain would also write Technical Notes, mark more characteristics with an asterisk (\*) and draw up a Technical Questionnaire.

#### Working Paper on Test Guidelines for Cotton (Revision)

38. The Working Party noted documents TG/88/3 and TWA/23/14 Rev. and made the following main changes to document TWA/23/14 Rev.:

(i) Subject of the Test Guidelines: To apply also to interspecific hybrids.

(ii) Table of Characteristics:

#### Characteristics

- 6 To be checked to establish whether it would be better to observe the third fruiting branch, and whether the proposed following characteristic should be replaced by the length of internodes
- 12 To be checked to establish whether a characteristic on the spot of the petal should be inserted after this characteristic
- 18 To be preceded by a characteristic on the presence of fuzz
- 22 To be checked to establish whether the second characteristic after characteristic 22 (Fiber: color) should have only the states "white, other color"
- To be checked to establish whether the intensity of gossypol should be included as a characteristic.

39. As limited time had been available for the study and discussion of the draft, the Working Party agreed to invite all experts to send any further comments on the document to Spain. The expert from Spain would then include Technical Notes and a Technical Questionnaire and produce a new draft, which would be discussed by UPOV experts on cotton attending the meeting on the EC comparative trials to be held in Greece.

#### Working Paper on Test Guidelines for Bromus

40. The Working Party noted document TWA/23/13 and the remarks from experts from France to the effect that, because of the different levels of self-fertilization in the species, the draft would propose the application of the COY analysis as practised so far for cross-fertilized species. The Working Party did not enter into detailed discussions of the document, but several experts did consider it dangerous, with a "minor crop," to start with changes in practice before having discussed whether the COY analysis could in principle be applied to self-fertilized crops, and also what the consequences of such application would be. The Working Party therefore agreed to postpone discussions on the establishing of Test Guidelines, and to deal first with the question of principle of the application of the COY analysis. It invited all

experts to send their comments on document TWA/23/13 to the experts from France, who would then prepare a revised draft for the next session.

41. In the meantime, it asked the TWC to make a comparison, on the basis of some real data to be supplied by experts from France, of the application of the present method for self-fertilized crops as set forth in document TWC/11/16 with the COY analysis as set forth in document TC/30/4. Thereafter, the consequences of application of the COY analysis to self-fertilized or mainly self-fertilized crops could be discussed on the basis of the two different results, and a well-informed decision could be taken.

#### Status of Test Guidelines

42. The Working Party agreed that the draft Test Guidelines for Wheat (Revision), Barley (Revision), Oats (Revision), Maize (Revision) and Fodder Beet should be sent to the Technical Committee for final adoption. It agreed that the draft Test Guidelines for Flax, Linseed (Revision) should be sent to the professional organizations for comments. It agreed to rediscuss the Test Guidelines for the other species mentioned on the agenda at its next session.

#### Future Program, Date and Place of Next Session

43. At the invitation of the expert from Germany, the Working Party agreed to hold its twenty-fourth session in Hanover, Germany, from June 20 to 22 (noon), 1995. The Working Party planned to discuss or rediscuss the following items at the session:

- (i) Report on the twenty-third session of the Working Party ;
- (ii) UPOV Central Computerized Data Base
- (iii) Survey on the use of electrophoresis;
  - Potato (Report from the Subgroup)
  - *Poa pratensis* (NL to prepare a document)
  - Ryegrass (GB to prepare a document)
  - Timothy (FR to prepare a document)
- (iv) Statistical methods (NL to prepare a document on ear rows/drilled plots, GB to combine documents TC/30/4 and TWC/11/16, advice from the TWC on the application of COY to self-fertilized crops);
- (v) Cooperation with breeders in the testing of varieties (UPOV to prepare a new questionnaire);
- (vi) Final discussion on draft Test Guidelines for:
  - Flax, Linseed (Revision) (TG/57/4(proj.))
- (vii) Discussion on working papers on Test Guidelines for:
  - (a) Rape (Revision) (TG/36/3, TWA/23/5 + report from Subgroup)
  - (b) Soya Bean (Revision) (TG/80/3, TWA/22/6, TWA/23/2 Rev.)
  - (c) Subterranean Clover (TWA/22/8, TWA/23/6)
  - (d) Rice (Revision) (TG/16/4, TWA/23/12 Rev. + ES to prepare a document)
  - (e) Cotton (Revision) (TG/88/3, TWA/23/14 Rev. + ES to prepare a document)
  - (f) Bromus (TWA/23/13 + FR to prepare a document)

44. The Working Party noted that a Subgroup on Potato would meet in Hanover, Germany, in November 1994 and a Subgroup on Rape in Versailles, France, at the beginning of 1995.

45. The Working Party noted that the Organization for Economic Co-operation and Development (OECD) would at its next session be discussing the question of new agricultural species on the list of species admitted for certification but for which no UPOV Test Guidelines yet existed. It also noted that, while the list was a rather long one, the number of species on it for which ten or more varieties were mentioned comprised only the following:

- Brassica juncea L. Czernj. et Cosson
- Brassica oleracea (Convar. Acephala) L.
- Sinapis alba L.
- Agrostis capillaris L.
- Arrhenatherum elatius (L.) P. Beauv. ex J.S. et K.B. Presl
- Bromus catharticus Vahl
- Bromus inermis Leysser
- Chloris gayana Kunth
- Phacelia tanacetifolia Benth
- Phalaris aquatica L. (incl. P. stenoptera Hackel, P. tuberosa L.)
- Sorghum bicolor X Sudanense
- Sorghum sudanense Stapf
- Lens culinaris Medikus (L. esculenta Moench)
- Lotus corniculatus L.
- Onobrychis viciifolia Scop. (O. sativa Lam.)
- Trifolium alexandrinum L.
- Trifolium hybridum L.
- Trifolium incarnatum L.
- Trifolium resupinatum L.
- Vicia villosa Roth
- Arachis hypogaea L.
- Cannabis sativa L.
- Papaver somniferum L.
- Trifolium subterraneum L.

All experts were invited to reflect on the question for which of the above species, or for what others in the complete OECD list, UPOV Test Guidelines should be planned. Those experts attending the coming OECD meeting were invited to report to the Working Party at its next session on the outcome of the OECD discussions on this subject.

46. The Working Party noted a preliminary invitation to hold its 1996 session in Thessaloniki, Greece.

#### Visits

47. On May 19, 1994, the Working Party visited the Technical Laboratory on Cotton Fiber in the Technical Institute for Tobacco in Seville, and also the testing fields at the INSPV Testing Centre in Coria, near Seville.

48. This report has been adopted by correspondence.

[Four annexes follow]

## ANNEX I

LIST OF PARTICIPANTS AT THE TWENTY-THIRD SESSION  
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Annex I, page 2

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[Annex II follows]

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## ANNEX II

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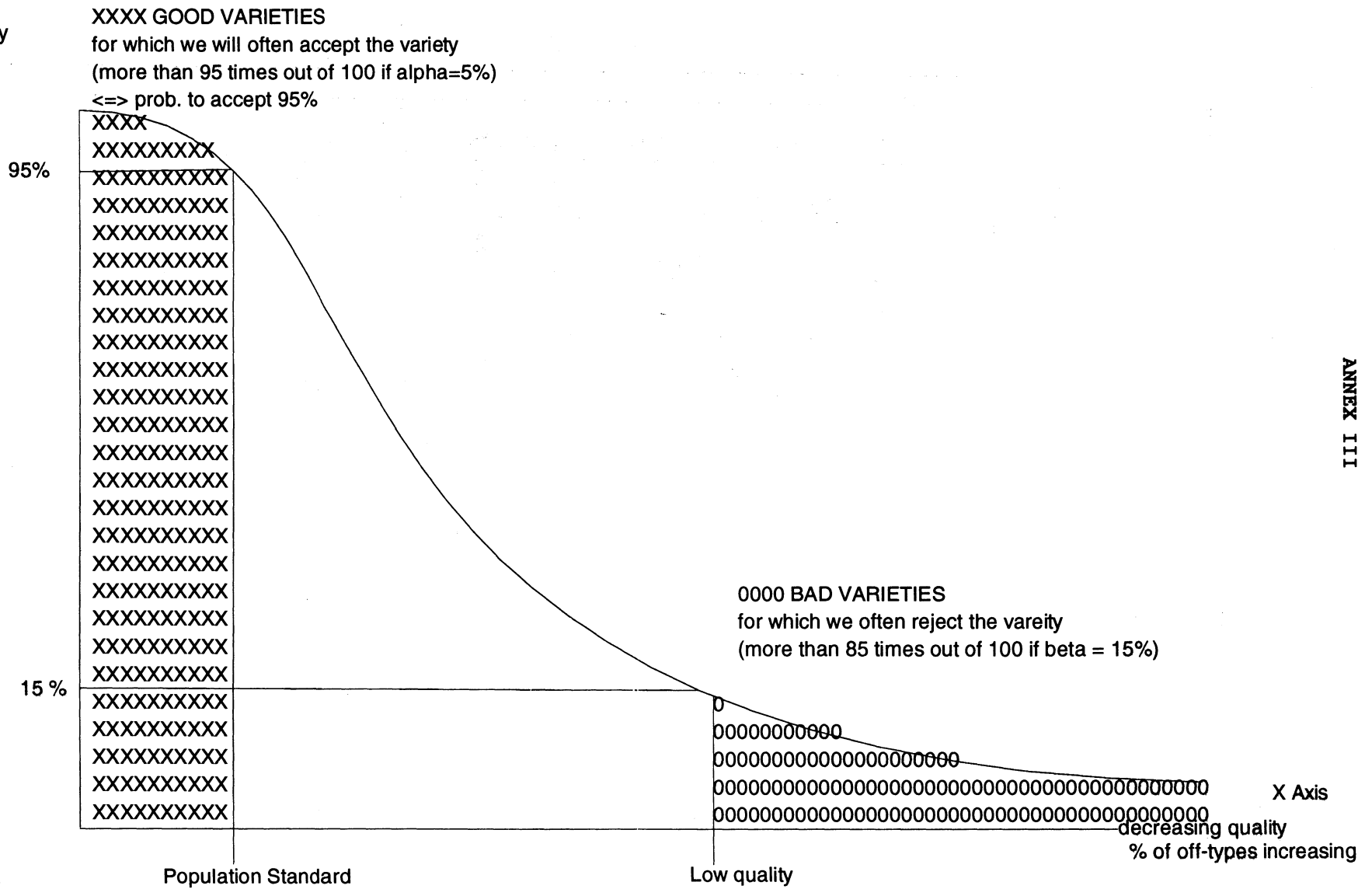
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[Annex III follows]

Y Axis  
probability  
to accept  
a variety



ANNEX III

TWA/23/16

Choices made

population standard 2.5 per 1000

alpha 5%

we usually looked to 1000 or 2000 plants.

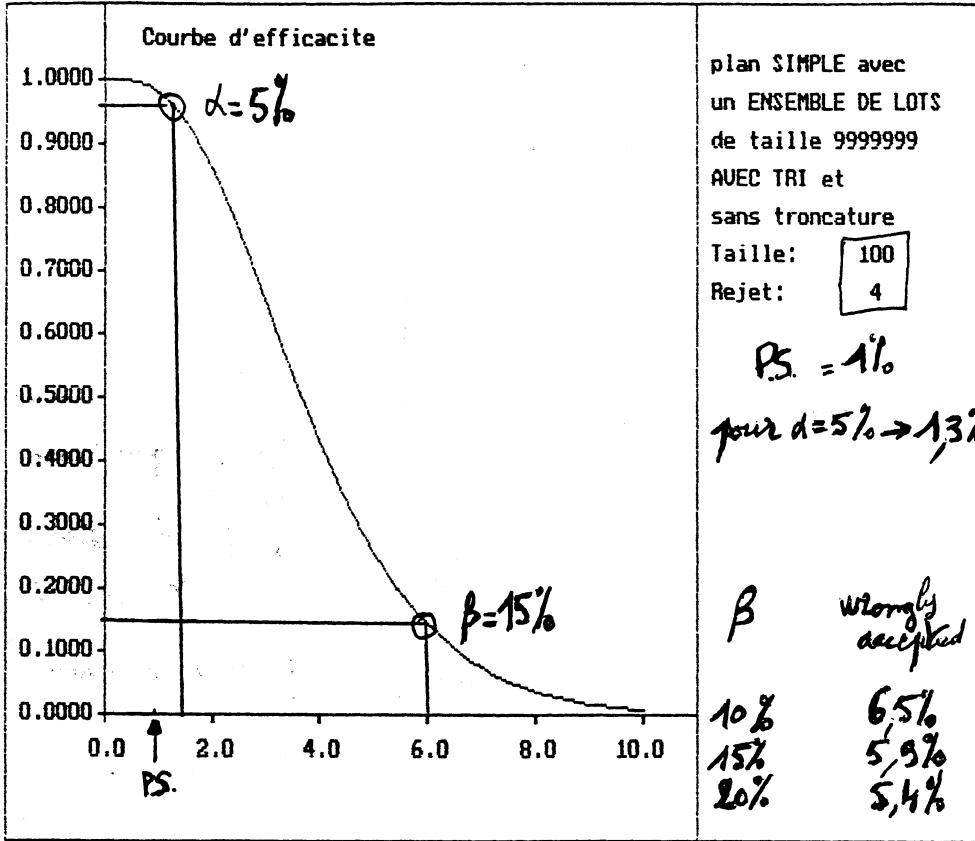
following schemes are appropriate:

if more plants are examined then the zone in which we are not satisfied (because we will accept wrongly heterogeneous varieties) is reduced.

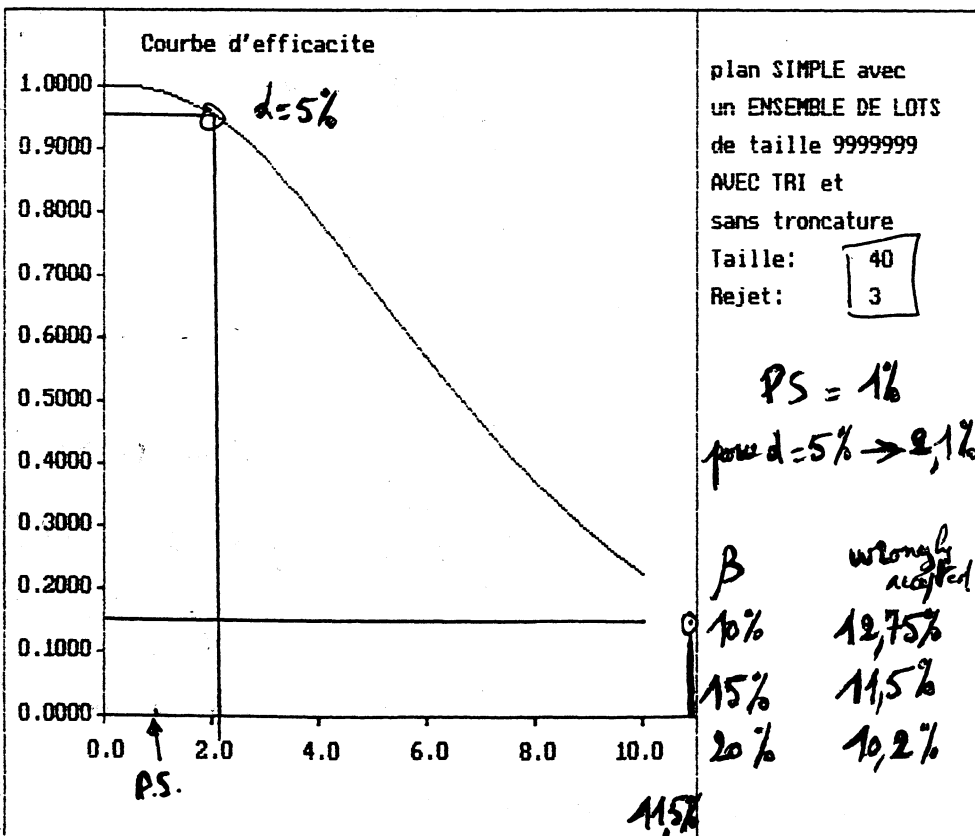
fixed at beginning	sample scheme appropriate	efficiency of the sample scheme
--------------------	---------------------------	---------------------------------

population standard	alpha	nb of plants to be examined	reject if more than this nb of off-types	beta =10%	beta=15%	beta=20%	"grey zone" where decisions less precise
2.5 per 1000	5%	545	3	12 per 1000	11 per 1000	10 per 1000	up to 4 PS
2.5 per 1000	5%	788	4	10 per 1000	9 per 1000	8 per 1000	
2.5 per 1000	5%	1044	5	9 per 1000	8 per 1000	7 per 1000	
2.5 per 1000	5%	1314	6	8 per 1000	7 per 1000	7 per 1000	up to 3 PS
2.5 per 1000	5%	1879	8	7 per 1000	6 per 1000	6 per 1000	
2.5 per 1000	5%	2171	9	6 per 1000	6 per 1000	6 per 1000	
2.5 per 1000	5%	4016	15	5 per 1000	5 per 1000	5 per 1000	up to 2 PS

ACTUAL BASIS OF TWC/11/16, TABLE 10  
Sample size 100 or 40



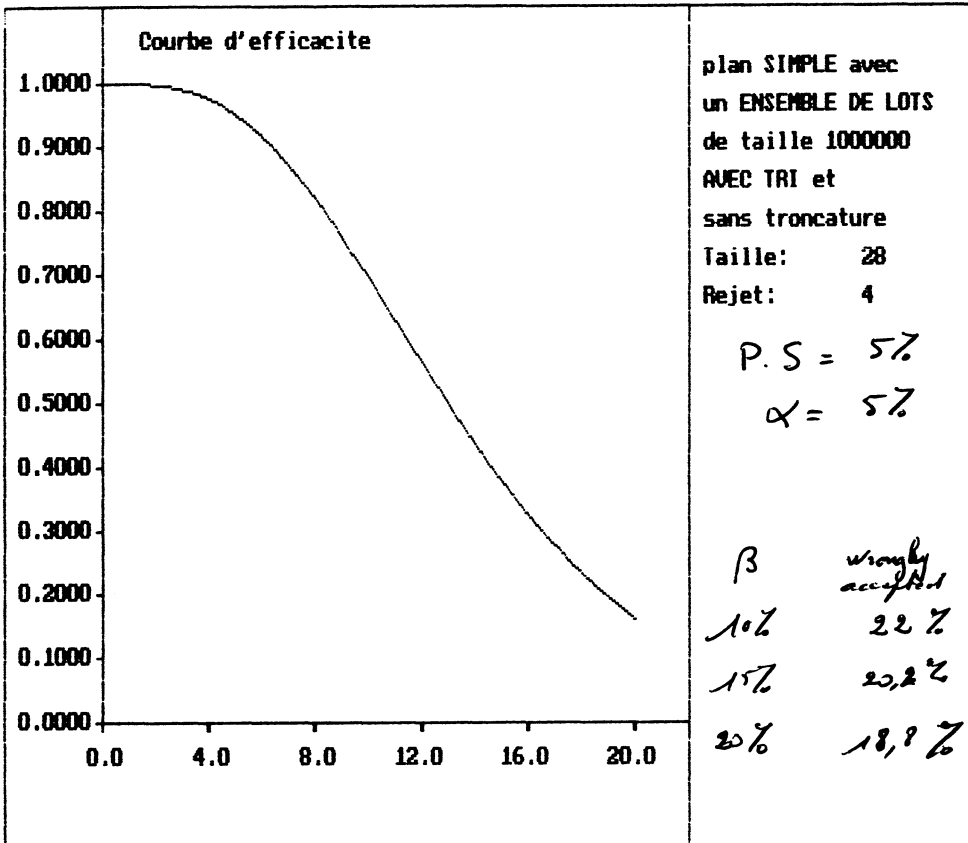
grey zone  
1,3% to 5,9%  
for  $\beta = 15\%$   
(about 6 times P.S.)



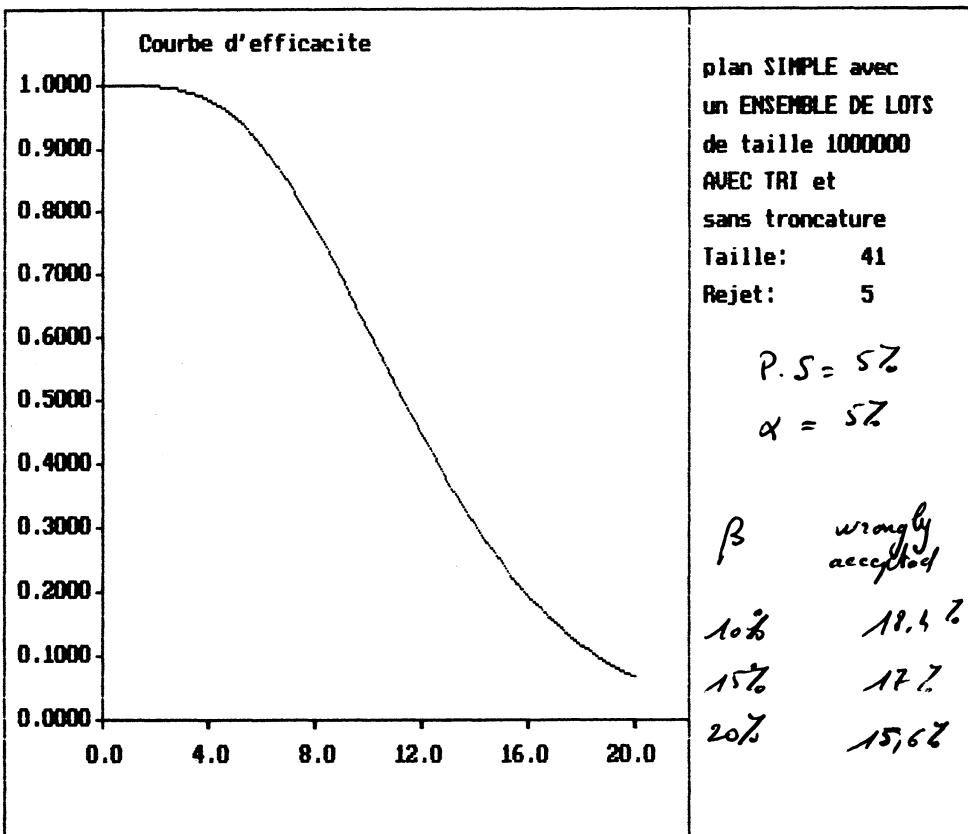
grey zone  
2,1% to 11,5%  
for  $\beta = 15\%$   
(about 11 times P.S.)



CASES WITH ALPHA = 5% AND LOW SAMPLE SIZES (28 OR 41)

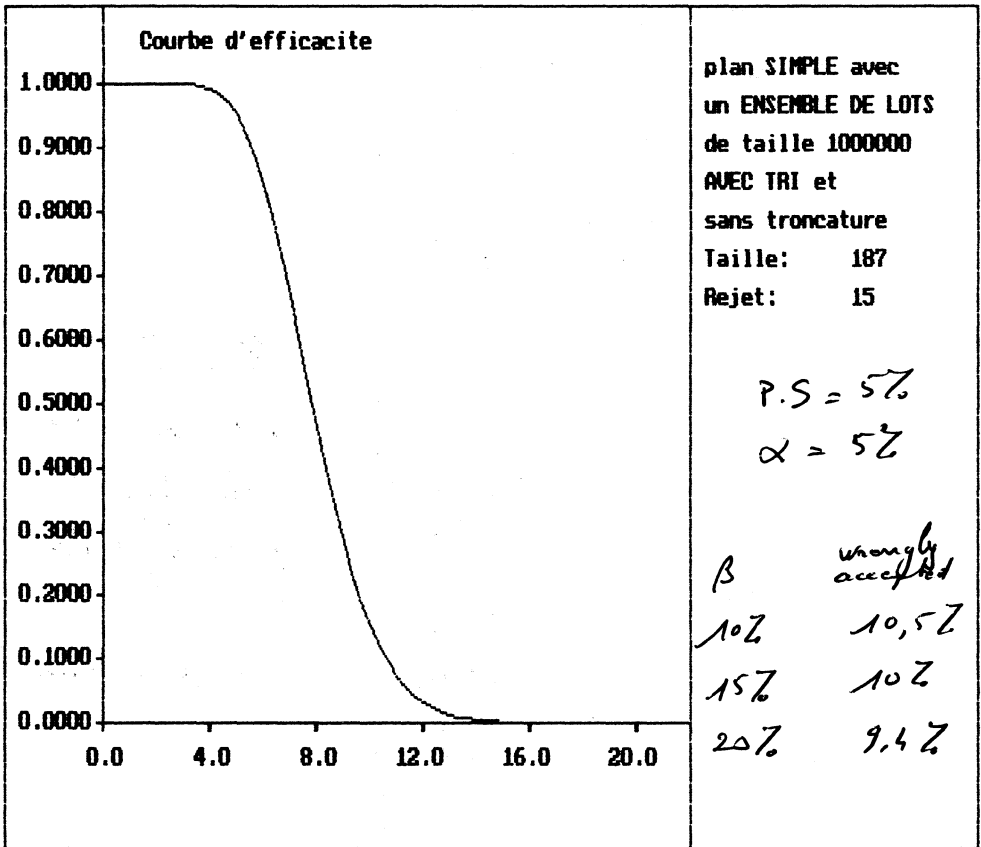


gray zone  
5% to 20,2%  
for  $\beta = 15%$   
(about 4 times P.S.)

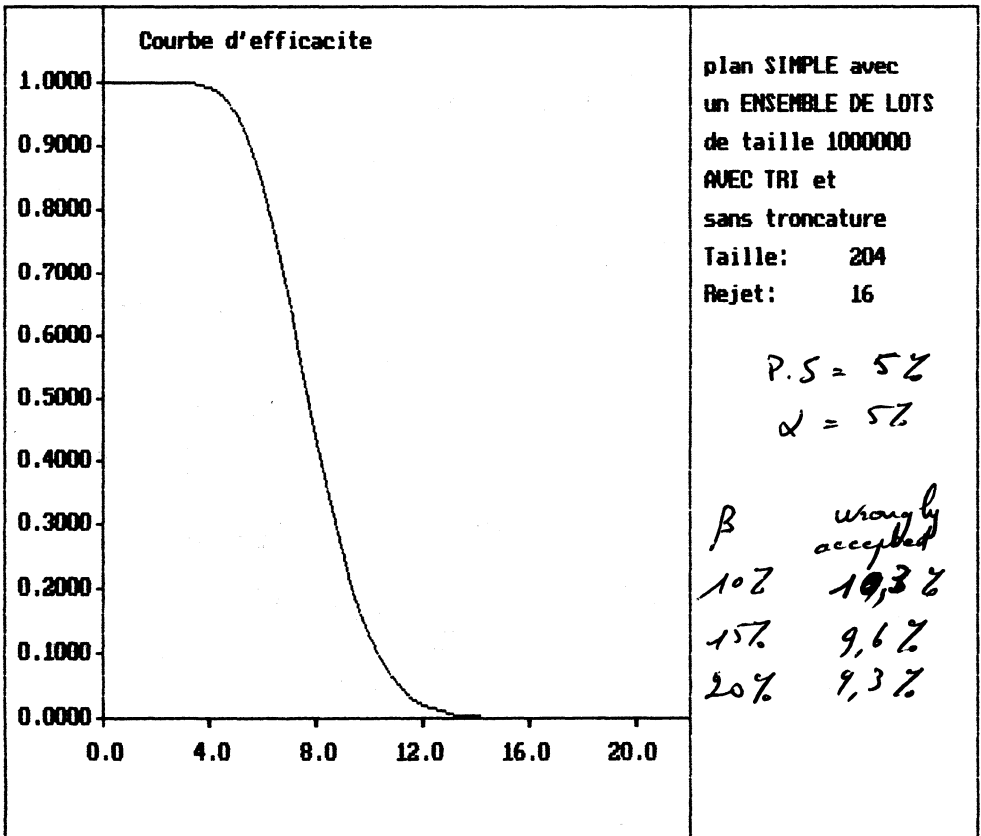


gray zone  
5% to 17%  
for  $\beta = 15%$   
(about 3 times P.S.)

CASES WITH ALPHA = 5% AND SAMPLE SIZES (187 OR 204)

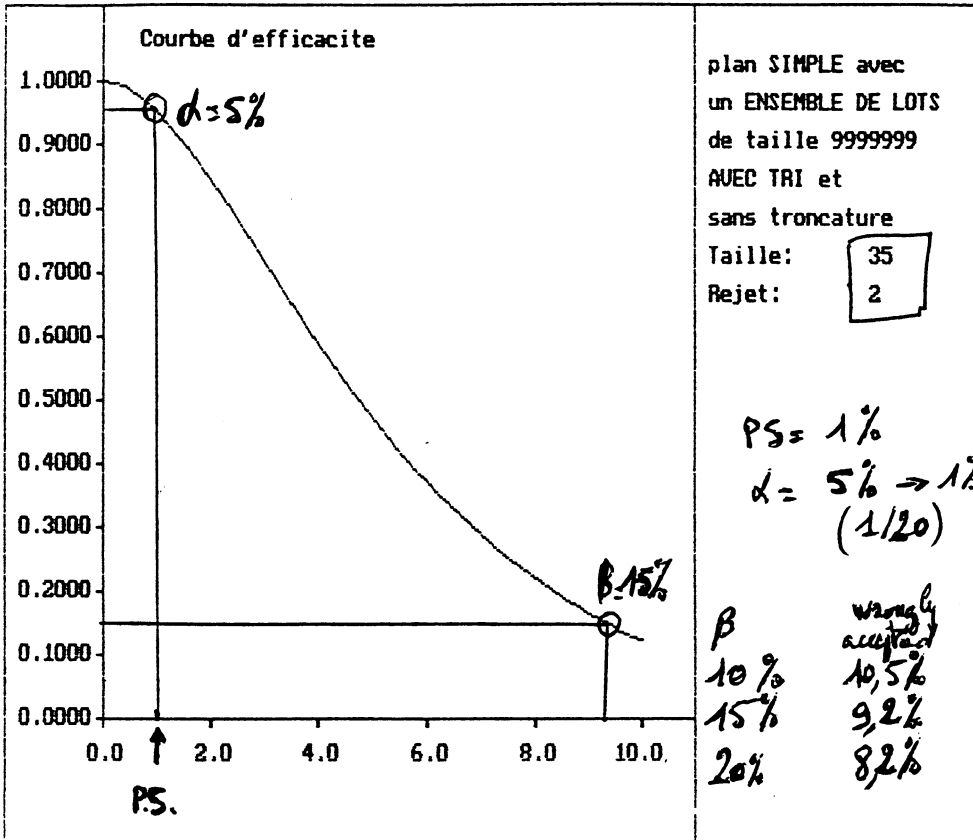


*gray zone  
5% to 10%  
for  $\beta = 15%$   
(about 2 times P.S.)*

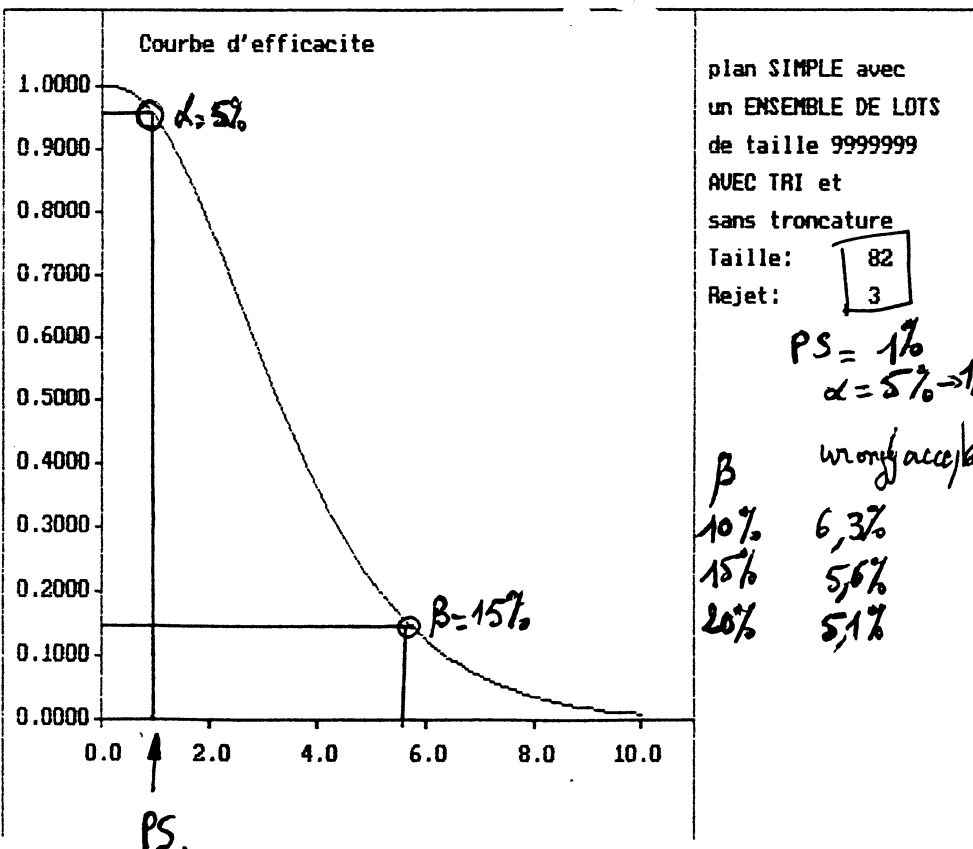


*gray zone  
5% to 9,6%  
for  $\beta = 15%$   
(about 2 times P.S.)*

MORE EFFICIENT CASES COMPARED TO THE HYPOTHESIS



grey zone  
12 to 9,2Z  
for  $\beta = 15\%$   
(about 9 times P.S.)



grey zone  
12 to 5,6Z  
for  $\beta = 15\%$   
(about 6 times P.S.)

La population standard doit être déterminée en fonction des objectifs, des normes de contrôle, de la biologie de la reproduction, de la génération, etc.

Ce n'est pas le dispositif expérimental qui dicte la population standard (ou alors de façon indirecte parce que l'on s'aperçoit que compte-tenu de l'effort qu'il faudrait fournir (nb de pltes à observer) il est impossible de fixer la population au niveau (bas) souhaité au départ).

On fait le test en regardant tous les caractères et non caractère par caractère.

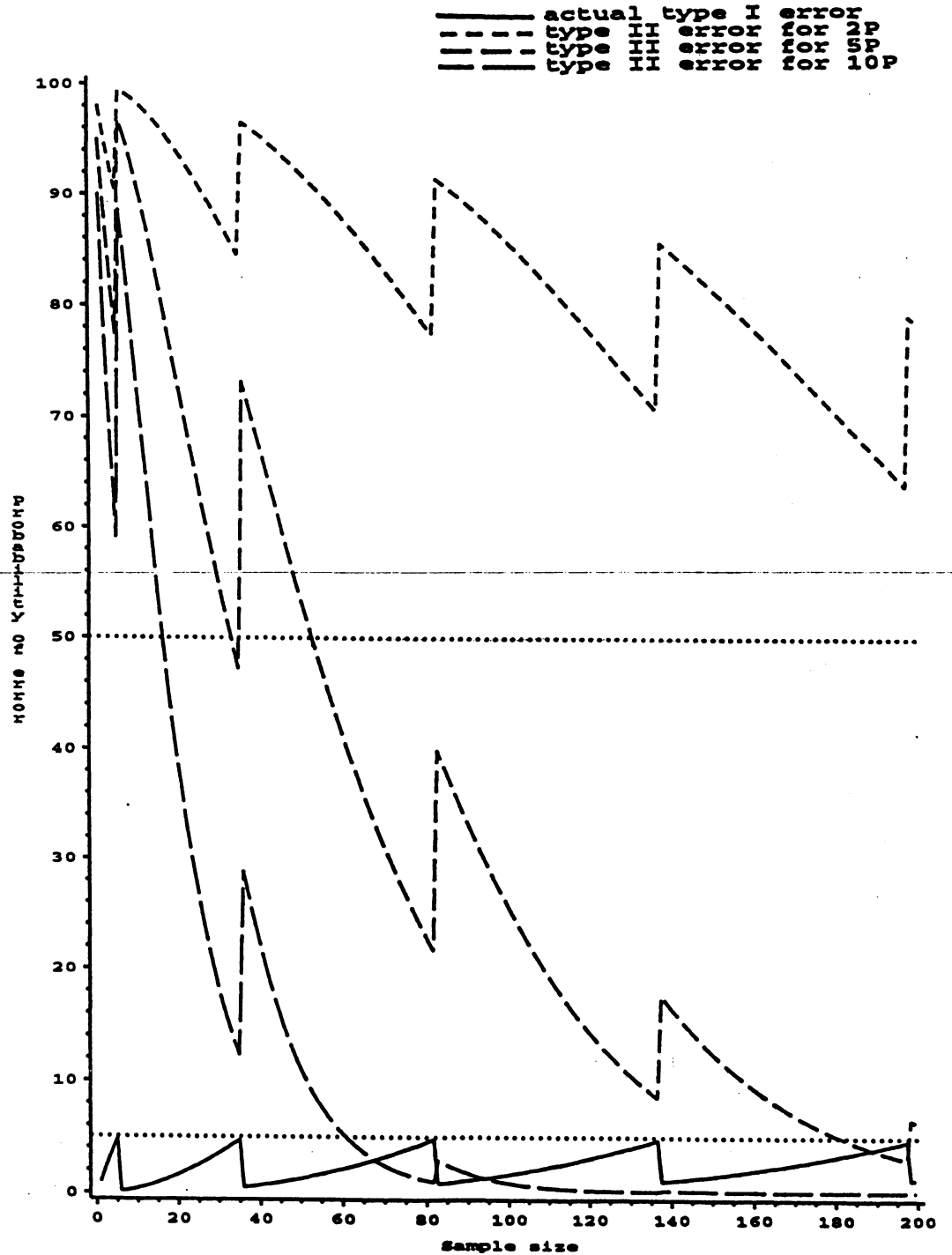
Les statistiques présentées jusqu'ici ne permettent pas de prendre en compte le fait qu'il est peut être plus ou moins difficile de reconnaître si c'est ou non un hors-type (précision...),

ni

que l'on a plus de chances de trouver des hors-types si on regarde plein de caractères que si on regarde un seul caractère.

**Table and figure 10: Population Standard = 1%  
Acceptance Probability  $\geq 95\%$   
n=sample size, k=maximum number of off-types**

n	k
1- 5	0
6- 35	1
36- 82	2
83- 137	3
138- 198	4
199- 262	5
263- 329	6
330- 399	7
400- 471	8
472- 544	9
545- 618	10
619- 694	11
695- 771	12
772- 848	13
849- 927	14
928-1006	15
1007-1085	16
1086-1166	17
1167-1246	18
1247-1328	19
1329-1410	20
1411-1492	21
1493-1575	22
1576-1658	23
1659-1741	24
1742-1825	25
1826-1909	26
1910-1993	27
1994-2078	28
2079-2163	29
2164-2248	30
2249-2333	31
2334-2419	32
2420-2505	33
2506-2591	34
2592-2677	35
2678-2763	36
2764-2850	37
2851-2937	38
2938-3000	39



[Source: document TWC/11/16, page 22]

[Annex IV follows]

TWA/23/16

## ANNEX IV

**REACTION OF PLANTS TO PESTS AND PATHOGENS**

Definitions proposed by the Scientific Committee of the CTPS of France and supported by the opinion of pathology specialists, October 1993

**Definition of the Terms describing the Reaction of Plants to Pests and Pathogens**

- The definitions below concern exclusively the specific host-parasite pairs between which there exists compatibility. They do not concern non-recognition between partners amounting to incompatibility.
- There exist differing degrees of specificity in the host-parasite relations. The identification of that specificity generally requires the use of highly elaborate analytical means.
- Recognizing whether a plant is subject or not to parasites may depend on the analytical method.
- It is important, in general, to stress that the specificity of pests or pathogens may vary over time and space and that new pathogen races or new pest biotypes capable of overcoming a resistance may emerge.

The following Terminology may be adopted in this context:

**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.

**METHODS FOR ASSESSING DISEASE RESISTANCE**

G. Doussinault, Scientific Committee of the CTPS, October 1993

**Methods for Assessing Disease Resistance**

Assessment of the level of plant resistance to diseases must be placed in the context of etiological and epidemiological studies.

A number of steps must be well understood:

1. Knowledge of the pathogen and its genetic variability resulting in the use of a perfectly known inoculum.
2. Inoculation and the disease development conditions.
3. Evaluation of the resistance level as a function of the age and physiological state of the plant by means of direct and indirect methods.

**Knowledge of the Pathogen**

Very similar symptoms may be caused by differing pathogens. Such is the case of foot rot and ear scab of wheat where two families of pathogens, Microdochium nivale and Fusarium sp., lead to very similar symptoms. The same applies to numerous viral diseases.

At intraspecific level, it is indispensable to know the virulence genes and the aggression level of the inoculum used.

The classic method is to carry out isolation, followed by identification. In order to go further, it is necessary to know the genetic structure of the inoculum (clone, population, etc.) and the virulence genes present in the genetic entity, by means of confrontation with a series of differential hosts. Considerable work remains to obtain knowledge and stability of the inoculums used in resistance analysis.

Moreover, recognition of the nucleic acids by means of specific initiators and the reproduction of nucleic acid markers for the genes involved in the pathogenic action by the use of the PCR technique may, in the long-term, assist significantly in obtaining knowledge of the inoculum that is to be used to carry out the virulence testing.

As an addition to other methods, the PCR techniques will probably make it possible to characterize an inoculum in a precise manner.

It is most important that once the inoculum has been characterized it should be maintained without modification and therefore strains that are cloned from a single haploid spore or viruses purified in plasmids or bacteria are used.

Reproduction of the inoculum may be effected on a nutritive medium in the case of necrotrophic parasites or on living plants in the case of obligate parasites. In the latter case, it is important that each strain of the pathogen be reproduced on the same universally susceptible plant genotype to ensure that the production conditions for the inoculum are comparable.

### Inoculation and Development Conditions

The host-parasite relations must be well-known in order to determine the conditions that are necessary to carry out repetitive testing and to reveal the interaction between the plant and the pathogen.

For example, in the case of pepper, certain resistance genes to Phytophthora capsici are active up to 22 degrees C but are not effective at 32 degrees C, whereas others are effective at both temperatures.

The development of the plant's resistance must be taken into account. Specific resistance genes for wheat powdery mildew are expressed as of the two-leaf stage, after which a non-specific resistance occurs that may be partly revealed following vernalization and which is fully expressed at the adult stage.

Inoculation may be effected naturally by contact with infectious particles by spraying onto the plant or by mixing with the culture medium.

It may also be done by force, by injection, grafting, dodder bridge (mycoplasma, certain viruses), agro-infection with cDNA or tungsten particle bombardment. In such a way, a resistance factor may be short-circuited. In the case of fungi that act through the intermediary of a toxin, that toxin can be used to measure the resistance level. Such is the case of wheat ear scab caused by Fusarium graminearum and F. culurarium of which the toxins can be used to measure the survival of wheat callus.

### Evaluation of the Resistance Level

The response to infection is sometimes of a qualitative nature, but is more frequently quantitative. It is necessary to be familiar with the epidemiology of a disease in order to characterize the various resistance factors that may emerge during the lifetime of the plant and the development process of the disease. It is necessary to reveal the various components capable of reducing the occurrence of the disease and to separately evaluate each of those elements.

Thus, resistance to powdery mildew in wheat measured by the significance of symptoms results from the effect of specific resistance genes that are expressed at the two-leaf stage and of a resistance that does not appear specific and that emerges progressively during the lifetime of the plant. In order to reveal the specific resistance genes, it is necessary to confront the various genotypes with a series of clones of the pathogen involved, Erysiphe graminis, having differing virulence genes and that enable the specific resistance genes and their associations to be revealed.

To assess resistance at the adult stage, it is necessary to confront the plant with a number of clones that possess the virulence genes capable of overriding the specific resistance genes present in the plant. Non-specific resistance is already partially installed after eight weeks of vernalization. Observations at that stage enable the resistance component that expresses in the adult stage to be forecast.

Palloix et al. were able to identify, through the examination of a large collection of genotypes in natural and artificial infection conditions, at least five elements of resistance that intervene at differing stages in the infection of pepper by the cucumber mosaic virus:



- partial resistance to transmission by vector aphids,
- a certain level of resistance to mechanical infection that limits the success frequency of inoculation,
- resistance to virus reproduction,
- resistance to virus migration through the plant,
- ability to display only weak symptoms, particularly in fruit.

Specific techniques have therefore to be developed to assess each component of resistance.

It is also possible to quantify the extent of the infection by serological tests in order to estimate the quantity of mycelium present in various parts of the plant.

Thus the study of pseudocercospora herpotrichoides has been undertaken in Germany by means of an ELISA test. The search for a specific fungus protein expressed in the infected plant constituted the preliminary stage to production of the serum.

Subsequently, the intensity of the ELISA test response, measured by absorbance of light, enables the quantity of mycelium present to be estimated since that intensity is an exponential function of the visual notation.

### Conclusion

In order to assess the resistance level of plants to disease, it is first necessary to analyze and control the virulence and aggressivity characteristics of the pathogen. The use of molecular biology should enable characterization to be more precise and more rapid.

It is then necessary to have full knowledge of the epidemiology of the disease in order to assess the resistance level at each stage in the development of the disease.

The resistance level may also be modified by the safeguard phenomenon and by the growing of mixed genotypes.

Indeed, there exist varying levels of tolerance for a given level of resistance.

Letter to the editor

## NOMENCLATURE FOR PATHOGENICITY AND VIRULENCE : THE NEED FOR PRECISION

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In a recent review, Shaner et al (9) considered the different meanings of terms related to pathogenicity as used in plant pathology literature and stressed the need for common designations of basic concepts. They proposed that avirulence and aggressiveness should be abandoned and replaced by nonpathogenicity and parasitic fitness, respectively. They also suggested a dichotomous hierarchy of terms describing pathogenicity, composed of virulence on one side and parasitic fitness on the other. The latter component was split further into specific pathogenicity and reproductive fitness. Finally, a quantitative meaning for virulence was proposed. Unfortunately, and as recognized by the authors themselves, no explicit definitions of these suggested designations are given, although the introductory sentence of the review rightly states that "precision of names given to concepts, structures and phenomena is indispensable to communication in science" (9).

In my opinion, the proposals suggested by Shaner et al (9) increase the confusion instead of clarifying the current nomenclature. The aim of this letter is to show that the concepts covered by the terms nonpathogenicity, avirulence, virulence, aggressiveness, and parasitic fitness all accept distinct definitions, and that they all are of value to plant pathologists for describing different realities.

**A virulent pathogens versus nonpathogens.** Part of the problem in accurately defining pathogenicity-related terms arises from the lack of a clear definition of what an infectious disease is. It is interesting to note that such a definition is absent from many major textbooks on plant pathology (e.g., 7, 15, 16), although it is the basis for all further concepts. The following discussion is restricted to infectious diseases, because physiological disorders do not imply the interaction of two organisms and, therefore, are outside the scope of the definitions of virulence, avirulence, aggressiveness, and parasitic fitness.

An infectious disease can be defined as a harmful alteration of the normal physiological state of an organism, called a host, due to challenge by another, called a pathogen. It results in "visible or otherwise detectable abnormalities," called symptoms (10). A pathogen succeeding in entering host tissue and deriving part or all of its nutritive substrates from it is called a parasite and is said to infect its host (10). The definition of a pathogen implies that a nonpathogen is an organism not inducing disease when challenging another (10); the challenged organism is then called a nonhost.

A major feature of nonhost resistance is that all members of the nonhost species are resistant to the nonpathogen (4, 6). The opposite extreme is when all members of a host species are susceptible to a pathogen. More commonly, only part of the genotypes constituting the host species are resistant to the pathogen; their resistance is called host resistance, and the pathogen is said to be avirulent to them. The challenge of a resistant host by an avirulent pathogen results in an incompatible reaction (6). On the other hand, the pathogen is said to be virulent to a host when it is able to infect it and, usually, to reproduce on it; the host is then said to be susceptible and the interaction compatible. These definitions make virulence and avirulence clearly qualitative traits, as used by Vanderplank (14, 15) and many subsequent authors (e.g., 1, 2, 5, 7, 11, 12).

It is clear that definitions of pathogenicity and virulence apply to pairs of organisms; therefore, one organism may be a pathogen for some species and not for others. A classic example is *Phytophthora infestans*, a pathogen of potato and tomato but not of wheat, citrus, or pines.

The definitions are simple to formulate, but recognition of the nonhost status of a resistant species is sometimes difficult. Niks (6) suggested that nonhost resistance is generally characterized by either avoidance mechanisms or by immunity, i.e., a high proportion of early abortion of the nonpathogen and infrequent necrosis of host cells. On the other hand, host resistance is most commonly expressed as either a hypersensitive reaction or necrosis associated with limited growth and reproduction of the pathogen (6). This led Tosa (13) to consider wheatgrass (*Agropyron repens*) as a host of *Erysiphe graminis* f. sp. *tritici*, because their interaction results in hypersensitive necroses of the plant cells. However, nonhost resistance is sometimes expressed mainly as necrotic reactions (6), showing that immunity cannot be equated with nonhost resistance. In spite of this difficulty in differentiating host from nonhost resistance, their separation into two different concepts is supported by mechanistic and genetic data. Day (3) suggested that a basic mechanistic difference existed between avirulence reactions, which are mechanisms of resistance "superimposed on an interaction that already has most if not all the elements needed for compatibility", and nonhost resistance, which is "generally due to failure to induce susceptibility". There also is accumulating evidence that the two types of plant resistance are mediated by different genetic systems (9).

**Aggressiveness versus parasitic fitness.** Although several meanings have been associated with the term aggressiveness in plant pathology literature (8,9), most plant pathologists currently use this term, as originally defined by Vanderplank (14,15), to designate the quantity of disease induced by a pathogenic strain on a susceptible host (e.g., 1, 7). From this definition, it appears that aggressiveness depends primarily on the pathogen but also on the host and the environmental conditions; it is, therefore, a characteristic of a host-pathogen interaction rather than of a pathogen alone. This appears clearly in a number of reports, showing that the same pathogenic isolate can induce different amounts of disease on a series of susceptible hosts (e.g., 7, 14, 15). There is, therefore, no such thing as "the aggressiveness of one strain" per se, measurable as the area of diseased tissue or the number of offspring produced by this strain on any susceptible host. Aggressiveness always depends on the partial resistance features of the host on which it is measured (15). Another attribute of aggressiveness is that it can be measured repeatedly in standard environmental conditions and is then a stable trait or for a given host-pathogen pair.

On the other hand, fitness classically designates the contribution of a given genotype to the gene pool of the next generation of the organism considered (9). Thus, parasitic fitness is basically an attribute of a pathogenic strain within a population, rather than of a single host-pathogen interaction. It obviously depends on the aggressiveness of the strain on the different hosts available for infection, as correctly outlined by Shaner et al (9), but also on the aggressiveness of the other genotypes composing the pathogen population on the same set of hosts. Because it is a result of the relative parasitic and reproductive abilities of a parasitic population on a host population, parasitic fitness of one pathogen strain is in practice not a stable trait, because populations and environmental conditions are never the same in two experiments. Consequently, the suggestion of Shaner et al (9) of equating aggressiveness and parasitic fitness is irrelevant, because these terms designate clearly distinct concepts.

The dichotomous separation of pathogenicity between virulence and parasitic fitness proposed by Shaner et al (9) also is a source of confusion, because the authors meant a quantitative definition of virulence. I cannot see where the separation is between a quantitative virulence (including, at least to some extent, the amount of disease produced by the pathogen) and parasitic fitness as equated to aggressiveness, because the latter term seems to be part of, but not separate from, the former. Although Shaner et al (9) stated that "we should not enforce dichotomies where none exist", they obviously did so by separating two overlapping concepts.

**Epidemiological implications.** Vanderplank (15) showed that race-specific resistance and race-nonspecific resistance have largely different epidemiological consequences, for the former delays the onset of the epidemic, whereas the latter reduces the rate of epidemic progression. Heath (4) stated that one of the most prominent features of nonhost resistance is the provision of a highly effective, durable protection of the nonhost plant. Therefore, the separation of pathogenicity both from the genetic and the epidemiological standpoints into three qualitative categories (nonpathogens, avirulent pathogens, and virulent pathogens) seems to be fully justified. Aggressiveness relates only to the latter qualitative category and depicts the amount of disease produced in a particular

susceptible host-parasite interaction. Parasitic fitness is a measure of the success of one pathogenic genotype in a given population challenging a range of hosts. Thus, these five concepts are markedly distinct, and the terms used to designate them, therefore, cannot be used interchangeably.

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