Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Dir96-01

Regulatory Directive

Guidelines for Efficacy Assessment of Fungicides, Bactericides and Nematicides

Applicants for registration are required to provide efficacy data to support the proposed use of their products. The data must be assessed to ensure that the product is effective for its intended purposes. General principles for efficacy assessment of chemical pesticides have been published as Regulatory Directive Dir93-07a.

This Regulatory Directive provides specific guidelines for assessing efficacy of chemicals for control of plant diseases caused by fungi, bacteria, phytoplasmas and mycoplasma-like organisms, and nematodes.

The Directive is based on Regulatory Proposal Pro95-02, dated August 8, 1995, with only minor modifications arising from the public consultations.

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5.1 Introduction

This document is Part 5 of the *Guidelines for Efficacy Assessment of Chemical Pesticides* (1). These guidelines outline general principles for testing the efficacy of various types of pesticides. A thorough understanding of Parts 1 and 2 of the *Guidelines*, especially the Test Procedures outlined in Section 2.1, is essential to using Part 5.

Reference:

(1) Anon. 1993. Guidelines for efficacy assessment of chemical pesticides. Agric. Can., Food Prod. Inspection Br., Plant Industry Directorate Regulatory Directive 93-07a, April 5, 1993. 12pp.

5.1.1 How to Use this Document

It should be noted that these are guidelines, not regulations, and scientific judgement should be exercised in their use.

General considerations, including the applicability of foreign data, are covered in Section 5.2; test procedures are amplified in Section 5.3. Evaluations of data are divided into two sections: in Section 5.4 assessment of disease control and crop tolerance are considered on the basis of individual trials; and in Section 5.5 suggestions are made regarding comparisons of different trials and evaluating overall results. In the final Section 5.6, more specific use patterns are given, organized by major disease or crop types; users need consult only the patterns that relate to their crop.

It is recommended that this document be used at each major stage leading to an application for major use registration of a pesticide. At the start, it may be used to assess the quality and quantity of the database, domestic and foreign (Sections 5.2,5.4,5.5). It may be used in planning a program of testing aimed at developing a registration application package (Sections 5.2,5.3), and in assessing the validity and usefulness of new data (Sections 5.4,5.5). Finally, it may be used to prepare the report supporting an application.

5.1.2 Scope

This document provides guidelines for assessing efficacy of chemicals for control of plant diseases caused by fungi (fungicides), bacteria, phytoplasmas and mycoplasma-like organisms (bactericides), and nematodes (nematicides). The guidelines do not apply to biocontrol agents, microbial products, and viricides.

5.2 General Aspects

The basic reference for this document is *Methods for Evaluating Pesticides for Control of Plant Pathogens* (2) with joint American and Canadian authorship. Another reference containing much applicable information is *Manual for Field Trials in Plant Protection* (3).

References:

(2) Hickey, K.D. (ed.) 1986. Methods for evaluating pesticides for control of plant pathogens. APS Press, Amer. Phytopath. Soc. 312 pp.

(3) Muller, G. and F.J. Schwinn (eds.) 1992 (3rd ed.). Manual for field trials in plant protection. CIBA-Geigy Ltd., Basle, Switz. 271 pp. (available from CIBA-Geigy).

5.2.1 Names and Terms

Common names of diseases and host plants, and scientific names of their causal agents should conform to usage in the *Compendium of Plant Diseases and Decay Fungi in Canada, 1960-1980* (6), *Names of Plant Diseases in Canada* (4), and *Guide to Plant Pathogenic Bacteria* (5). For scientific names of pathogens both the genus and species names are given, except where several species in the same genus produce similar symptoms and damage, e.g., damping-off caused by *Pythium* spp.

Definitions of plant disease assessment terms are given by Nutter *et al*. (7,8).

References:

(4) Anon. 1992. Names of plant diseases in Canada. Que. Soc. Protection of Plants.

(5) Bradbury, J.F. 1986. Guide to plant pathogenic bacteria. C.A.B. international Mycol. Inst., Kew, U.K. 332 pp.

(6) Ginns, J.H. 1986. Compendium of plant disease and decay fungi in Canada 1960-1980. Publ. 1813, Res.Br., Agric. Can. 416 pp.

(7) Nutter, F.W. Jr., P.S. Teng, F.M. Shokes. 1991. Disease Assessment terms and concepts. Plant Dis. 75: 1187-1188.

(8) Nutter, F.W. Jr., P.S. Teng, M.H. Royer. 1993. Terms and concepts for yield, crop loss, and disease thresholds. Plant Dis. 77: 211-215.

5.2.2 Label Claims for Plant Disease Control

Each activity that the registrant wishes to put on the label as a claim must be supported by scientifically acceptable information. Label claims cannot be justified by anecdotal or testimonial evidence alone that a product is effective, although such evidence may be used to support scientific data.

To be considered for registration, the product should provide a level of disease control that would result in a demonstrated benefit to the user.

5.2.3 Crop Development Stages

Where crop development stages are given as part of the instructions for timing of chemical applications (see Section 5.3.6), standardized keys for growth stages should be used wherever possible. The new BBCH universal decimal code, jointly developed by BASF, BAYER, CIBA-Geigy and HOECHST (9), is strongly recommended. The BBCH scale provides a universal description of phenologically equivalent growth stages of crops, including woody plants and weeds; it was developed from Zadok's scale to apply to all crops, and for computer use.

For labelling, crop development stages should also be given in descriptive terms.

Reference:

(9) Lancashire, P.D., H. Bleiholder, T.v.d. Boom, P. Langeluddeke, R. Strauss, E. Weber, and A. Witzenberger. 1991. A uniform decimal code for growth stages of crops and weeds. Ann. Appl. Biol. 119: 561-601.

N.B. The CIBA-Geigy Manual (3) also describes and illustrates this scale.

5.2.4 Non-Canadian Data

Where practicable, registration application packages should be based on original Canadian data. However, efficacy data from other countries may provide the starting point for testing a pesticide under Canadian conditions. Registrants should consult with the Product Manager for the number of Canadian trials required. Foreign data collected under conditions comparable to intended Canadian use situations which meet Canadian requirements may be used in support of Canadian registrations, particularly data from controlled environment studies.

5.2.5 Interactions and Disease Complexes

Some disease symptoms may be caused by more than one microorganism acting independently, or by two or more organisms acting in concert, by pathogens acting in sequence, e.g., damping-off, and by micro-organisms in seasonal sequences, e.g., crown rots of alfalfa. In other cases, a mild infection by one organism may either predispose a host to infection by another destructive pathogen, or, contrarily, may provide cross-protection against a second pathogen.

In all cases, label claims must specify, and registration applications must demonstrate through laboratory and/or field studies, which organisms were controlled. Control of the whole disease complex cannot be claimed unless all principal components were tested both individually and together.

Where causal agents of disease are vectored by another organism, control of the disease by controlling the vector is dealt with under the part of these guidelines appropriate to the vector, e.g., insect vectors are covered by Part 5.

5.2.6 Components of Efficacy

Several parameters may be used to measure effectiveness of chemicals in controlling disease. Direct measurements of disease include the number of plants infected, symptom severity per plant or plot, changes in symptoms, and plant survival. Indirect parameters include height, dry weight and gross yield; and quality factors such as appearance of seeds or fruit, storage characteristics, and marketable yield; and pathogen populations. Two or more factors may be combined into a disease index. When in doubt as to what factors to use, consult the Product Manager.

Registrants should show that there was an increase in crop yield and/or quality using both direct and indirect measures, and that this was due to control of the disease claimed.

5.2.7 Pesticide Tolerance of Crops

The applicant for registration should establish pesticide dosage rates within which the host may be safely treated, and give evidence of the symptoms expressed when these rates are exceeded. Since tolerance usually differs with growth stage, safe dosages must be established for the stages to which the chemicals are to be applied (see Section 5.4.3 for further guidelines). Where a formulation may be used on crop combinations such as pasture mixes, companion or nurse crops, tolerance of the associated crop(s) should also be established before label claims can be added.

5.2.8 Bridging Data and Comparability Tests

Efficacy claims for new formulations, and label amendments to add other types of the given crop species or other cultivars, may only require "bridging" data rather than the comprehensive information required for an original registration (see Section 2.1.15). For example, if Argentine rapeseed/canola is already on the label and there is no indication of a pesticide tolerance problem, confirmatory trials at a few representative locations may be sufficient to add Polish rapeseed/canola to the label.

It is recommended that the applicant consult with the Product Manager before embarking on such trials.

5.3 Test Procedures

5.3.1 Test Substances

The test substance shall be the formulated product for which registration is sought (see Section 2.1.2). The test substance shall be compared to check treatments, and if possible, a reference product. The reference product should be the most widely accepted product, in current commercial use, for controlling the same disease. Where two or more products are widely used, any of them may be used as the reference product.

Any substances added at application, such as adjuvants, coatings and adhesives, should first be tested separately by the registrant. Provided that no problems are found, all these ingredients should be combined with the active ingredient(s) into a single treatment for subsequent tests, and for tests conducted by independent researchers.

When available, the mode of action and rate of breakdown of the test substance should be indicated to independent researchers and in the submission since they affect the timing and frequency of pesticide application.

5.3.2 Check Treatments and Representative Cultivars

For a test to be scientifically valid, pesticide treatments must be compared to one or more check treatments. These include: (a) an untreated check; and (b) an efficacy check or reference product when available.

One or more of the most widely used commercial cultivars must be used. It is also desirable, because of varying degrees of resistance in commercial cultivars, to include both susceptible and resistant cultivars in some tests when available.

5.3.3 Dosage

The pesticide should be tested at various dosage levels, including levels above and below those being suggested for commercial use (see Section 2.1.3). Development of a dosage range serves to determine the minimum and maximum recommended dose. The label should indicate the timing and rates of application. Dose ranges are also used to establish the likelihood of phytotoxic effects on the crop. The label should differentiate the dose range appropriate to a once-in-a-season application from the dose range suitable for multiple applications, and state the maximum total amount that may be applied in a season.

If necessary, consult with the Product Manager to determine the range of doses which should be tested.

5.3.4 Selection of Trial Sites and Plot Sizes

Climatic conditions, soil type and conditions, and agricultural practices may have a significant influence on the effectiveness of a pesticide (see Section 2.1.4). Tests should be conducted at sites representative of the major conditions under which the pesticide will be used. The number of tests required depends on each crop/disease combination. Choice and number of test sites required also vary with each crop/disease combination. When in doubt, consult the Product Manager.

In controlled environments the principal variants to be quantified are growth substrates, temperature, light, humidity and wetness periods. In controlled storages, airflow and concentration of atmospheric gas are also principal factors to be measured. Controlled environment tests cannot be used exclusively to support field use registrations; however, they are useful in preliminary screening of candidate pesticides, selection of one or more pesticides for extensive field testing, and providing supportive data.

Controlled environment tests may be used, or even required, when there is danger of a strain that is more virulent than endemic populations escaping from plots. In such cases, pesticide tolerance tests may still be carried out in the field as they do not require the presence of the pathogen. Controlled environment tests are required for controlled environment use registrations, e.g., greenhouses, mushroom houses.

To reduce drift from one treatment to another, add untreated rows between treatments to the experimental design. These rows may be of a taller or denser cultivar, or a more disease-resistant or spray-tolerant species. Physical barriers such as plastic sheets can also be used. Plots may also be planted farther apart to provide a distance break.

Plot sizes (see Sections 2.1.5 and 2.1.6) selected vary with the crop/disease combination being tested. For practical reasons, plots are usually as small as can be while still obtaining adequate disease development and control. The larger the plot is, the less the interplot interference. On the other hand, the larger the disease difference between adjacent plots, the greater the interplot interference, and the more replicates needed. There is also a normal progression as product testing continues and the range of desirable options is narrowed: from controlled environments, to nursery or small field plots, to large plots. Sub-sampling techniques are appropriate for assessment of large-scale testing.

5.3.5 Disease Pressure

It is essential to ensure adequate disease pressure for tests to give useful efficacy data. It is desirable that disease levels in the check treatment be within the range usually found in commercial crops.

High inoculum levels may be required for development of disease to a point where pesticide efficacy can be adequately tested. When using natural inoculum, judicious site selection and

testing of inoculum levels are required prior to testing. Use of natural inoculum may also require larger sites accommodating more replicates to account for variability in inoculum levels across the plot area. Alternatively, very small plots or numbers of treatments may be used so that a test can be fitted into an area where inoculum levels are uniform. Alternate strips of a susceptible cultivar can also be used to maintain uniform inoculum among plots for polycyclic diseases.

Artificial inoculum may be used to overcome these field problems. Inoculation methods include scattering naturally-infected plant debris or foliage, or artificially produced propagules in plots; spraying spores collected from infected fields or artificial growth media; and injecting a pathogen into plant parts directly. In controlled environments, or where particular strains of a pathogen must be used, artificial inoculation is usually employed. Special precautions must be used when inoculating with strains not present at the test site in case of escape, especially with virulent strains or exotic pathogens.

Where necessary, provisions should be made to attain and maintain environmental conditions conducive to uniform disease development. These may include selection of appropriate test sites, irrigation, misting, or provision of row covers. Otherwise, the test site should be managed according to accepted commercial practices whenever feasible.

Disease complexes are best tested where they occur naturally as they are usually very difficult to reproduce artificially. Testing over several years may be required to obtain a sufficient number of valid tests.

5.3.6 Timing and Frequency of Applications and Pesticide Combinations

The effectiveness of a pesticide can often be influenced by the way it is applied (see Section 2.1.7 on application techniques). In addition, the timing of applications is often critical, and may refer to the season, growth stage, pre-harvest period, or time of day. It may also refer to periods of spore release by the pathogen; or to duration of weather conducive to infection of the host.

The label should indicate the season or growth stages in which application is effective, the maximum number of applications that may be used in any growing season, and either the last growth stage at which application is required or the minimum pre-harvest interval in days. Timing of applications should be indicated by growth stages (see section 5.2.3) rather than calendar dates or months, because dates at which particular growth stages are reached vary by location and between seasons.

The frequency and maximum number of pesticide applications depend on crop tolerance of the pesticide, life cycle of the pathogen, induction of pathogen resistance with repeated use, duration of residual activity of the pesticide, and requirements for efficacy. The pre-harvest

interval, however, depends primarily on the speed of breakdown or dissipation following application.

Both weather-related and time-of-day recommendations are based on periods of host susceptibility and exposure to infection, e.g., duration of periods of optimal relative humidity, dew period, leaf wetness, or diurnal periodicity of spore release.

Prediction systems have been developed for some diseases based on quantification of such factors, e.g., late blight of potatoes, apple scab, and onion leaf blight, and should be used in efficacy tests when available.

Pesticide combinations refer to application of two or more products. Combinations may occur in manufacturers' formulations, tank-mixes, concurrent applications from separate spray tanks (see Section 2.1.13), or sequential applications of one or more pesticides (see Section 2.1.14). When selecting pesticides for a combination, choose two or more pesticides with different modes of action, such as a single-site with a multi-site inhibitor, different sites of action, or in alternating sequence. The pH should be measured after mixing. Testing of the combination is required before it can be added to a label. Bridging data from comparison trials may suffice, however, as each active chemical in the combination will have been tested separately in most cases. These comparison trials should demonstrate the physical compatibility of the combination's components, with no side effects, and no new or enhanced phytotoxicity. The Product Manager should be consulted.

For combinations with other types of pesticides, such as insecticides or repellents, each pesticide type in the combination should conform to guidelines for that type of pesticide.

Sequential applications may involve repetitions of a single product, a combination, or different products aimed at the same pathogen. Repetitive applications may be required when there are recurring conditions conducive to new infections, or when the first application failed to control the disease. Possible problems that occur with sequential applications include increased phytotoxicity and selection of pathogen strains resistant to the treatment. The former may require a series of reduced dosages, and the latter the development of new combinations or alternatives with products having different modes of action.

5.3.7 Pesticides Used with Fertilizers and Inoculants

Pesticides may be used in combination with fertilizers (see Section 2.1.17) or inoculants. Inoculants are classed as fertilizers for regulatory purposes, and therefore also come under the *Fertilizers Act and Regulations*. All classes of components should be tested both separately and together and shown to be compatible and beneficial. Such components must be registered individually. Consult the Product Manager to ensure that sufficient types of tests are carried out.

5.3.8 Experimental Design

Efficacy tests must be designed so that valid and appropriate statistical analyses of the data can be conducted (see Section 2.1.1). All the factors considered thus far in Part 5 should be taken into consideration in designing efficacy trials (10). A series of trials, each one testing the range of a single factor, is usually simpler than mixing several factors, although the latter may be more economical and is the only way to show interactions among factors.

Test objectives should be clearly defined and should include only treatments aimed at those objectives, plus adequate checks (see Section 5.3.2).

The number of replicates used is usually a compromise between the statistical optimum and either feasible plot size or attainable uniformity, e.g., soils tend to be more variable the larger the plot. There are methods for calculating the minimum number of replicates needed to detect differences between treatments under different circumstances (see Nelson (10) and the textbooks he cites). Four replicates is the number most often used. With a larger number of replicates the results from one trial are more likely to be reproducible in another. In multifactor experiments, some of the replication of a treatment level may be internal, thereby reducing the need for external replication. Everything feasible should be done to make a trial as uniform as possible.

For suitable trial layouts consult a statistician or a compendium of appropriate experimental designs (10).

References:

(10) Nelson, L.A. 1986. Use of statistics in planning, data analysis, land interpretation of fungicide and nematicide tests. Pp. 11-23 *in:* K.D. Hickey (ed.) *op. cit.*

5.4 Evaluation of the Individual Trial

Each trial must be fully evaluated, analyzed, and conclusions drawn before it can be used as a result for comparison with other trials. It must be judged to be a valid test with adequate disease pressure, be free from extraneous interferences, and should be subjected to appropriate statistical analyses before it can be used in further evaluations to support an application for registration.

5.4.1 Validity of the Test

The results of all valid tests conducted under relevant conditions should be presented in the registration package.

A valid test is one in which there is adequate disease pressure and where there is no *a priori* reason to discard the data. Some ramifications of these statements are indicated below.

To determine whether a disease control test is valid, a number of factors should be measured or taken into consideration, including measurement of disease pressure (see Sections 5.2.7 and 5.3.5), observations on weather and other external influences.

The principal determinant of whether there is sufficient disease to assess pesticide effects is the level obtained in the check plots. Disease levels may be measured at the time of pesticide application, and at intervals up to harvest. The number of measurements will depend on the number of major growth stages and stages of disease development between application and harvest, and whether symptoms can be detected after onset of senescence.

A priori reasons for discarding the data may include weather factors. Apart from obviously catastrophic events such as hail, hurricane, flood, and killing frost (all of which can cause a test to be terminated and/or the data discarded), weather can have other significant effects on crops and data, e.g., a slight frost may cause significant damage at blossom, and yet this damage may be undetectable two weeks later or after fruit drop. Daily weather monitoring may be required within trials at critical periods. If so, sensors should be placed where the disease control treatment is actually being tested, e.g., amongst the seeds in the soil for a seed treatment test, or at branch level for a tree fruit. A set of reference sensors should be placed in a standard Stevenson screen, so that weather data collected may be compared to official weather records.

Records should be kept to indicate whether host establishment and development were normal, and all aberrations should be noted. Where plots have been influenced by an extraneous factor, they should be discarded, and corrective statistical techniques for missing data applied.

Where disease development is uneven through a plot, this should be noted and if possible mapped in order to identify a significant source of variability, or handled in blocked designs. Care should be taken to select correct methods of assessment.

A part of validation of a trial may include evidence that the test substance reached the target (specific parts of the plant), e.g., by using fluorescent dyes, water-sensitive paper, or other appropriate means (3).

5.4.2 Statistical Analyses

All valid tests should be subjected to statistical analyses appropriate to the experimental design and to the responses measured (10), (see Section 2.1.11). Each test should be properly analysed and conclusions drawn before comparing it to other tests.

For example, response data should be analyzed according to their distributional properties, i.e., if the variances are homogeneous, or are of Poisson-type, or binomial-type, or have some other distributional pattern, the appropriate procedure should be adopted. The effectiveness of pesticides can be quantified by fitting dose-response curves to the data (10,13).

The analysis of variance (ANOVA) and ordinary least-squares regression analysis are applicable only to continuous responses which do not have either a theoretical lower or upper bound (or both). These familiar analysis procedures are inappropriate to discontinuous data, such as counts, number surviving out of the number exposed, as well as other classes. Similarly, multiple range tests are appropriate only when there is no structure amongst the treatments (13), and certain multiple range tests may no longer be considered sufficiently rigorous for some situations. Other procedures, such as the Waller-Duncan Bayesian test, “protected” LSD, or orthogonal contrasts may be used under appropriate circumstances (10,11,13,14, 15, 16).

It should be noted that significance at the 5% level or lower ($P<0.05$ or $P=0.05$), although arbitrary, is the standard used for significance in science. Other standards, however, may sometimes be used (12).

References:

(11) Baker, R.J. 1980. Multiple comparison tests. Can. J. Plant Sci. 60:325-327.

(12) Little, T.M. and F.J. Hills. 1978. Pp. 24-25 *in:* Agricultural Experimentation. J. Wiley and Sons, N.Y. 350 pp.

(13) Little, T.M. 1981. Interpretation and presentation of results. Hortsci. 16:637-640.

(14) Swallow, W.H. 1984. Those overworked and oft-misused mean separation procedures --Duncan's, LSD, etc. Plant Dis. 68:919-921.

(15) Steel, R.G.D. and Torrie, J.H. 1960. Principles and Procedures of Statistics. 2nd ed. McGraw-Hill, New York.

(16) Snedecor, G.W. and Cochran, W.G. 1980. Statistical Methods. 7th ed. The Iowa State University Press, Ames, Iowa. 507 pp.

5.4.3 Assessment of Pesticide Tolerance of Crops

The second part of an assessment of a pesticide's performance is to evaluate what injury it does to the host plant (phytotoxicity), if any (see Section 2.1.10,b). When no damage to the host is found at recommended dosage levels, it is desirable to test the pesticide at higher rates to establish a margin of safety between the maximum recommended dose and the onset of phytotoxicity.

Any injury or abnormality should be recorded and the extent measured. Measures such as percent area damaged, and reduction in plant height, weight or yield should be used. Any deformations, changes in color, number of stems or leaves, and delays in development and maturation should be noted.

When phytotoxic responses are noted, their duration and effects should be measured, e.g., phytotoxicity may be short-lived and have no effect on yield or quality, or there may be a net benefit from pesticide treatment despite phytotoxicity, or the losses may require a change in dose or pesticide.

Finally, there may be differences in tolerance to the pesticide amongst cultivars of a given crop. All submissions should include tests on representative cultivars currently in commercial use.

5.5 Evaluation of the Pesticide

Conclusions should be drawn on the efficacy of a pesticide based on the results from a series of trials (see Sections 2.1.11 and 2.3). Data from all valid tests should be used to draw conclusions.

5.5.1 Comparisons of Different Trials

Data from similar tests can be analyzed to determine their similarity or dissimilarity. In comparing trials, allowance has to be made for non-comparable factors such as different soils, weather, inoculum levels, spray deposition and agricultural practices. Factors that may be compared include standard and check treatments, cultivars, experimental design and methods, and inoculation procedures.

Submissions should include reports on trials conducted by all agencies including the manufacturer's trials, and private and public researchers. All published results should be included.

Results of different trials should be presented in summary tables. Individual trial results should be attached in an appendix and numbered. Conclusions should be drawn clearly, and the most desirable application procedures, dose ranges, timing, and limitations indicated. Each conclusion and label claim should be supported by the data presented.

5.5.2 Report Format

See Section 2.2, Report of Efficacy Data.

Each report should clearly reference the diseases, pathogens and product which will appear on the proposed product label. All relevant information listed in Section 2.2.3 of these Guidelines should be included. Additional information which is important for disease control products includes:

a) inoculum source and method of inoculation (natural, artificial);
b) method of disease assessment (e.g., percent leaf area with lesions); and
c) identified checks as described in Section 5.3.2

See Table 1. Summary of field data required.

For reports of individual trials, the Pest Management Research Report format may serve as a model.

5.6 Specific Use Patterns

5.6.1 General

Evaluators should have a clear understanding of the type of chemical control that they are trying to obtain. This will depend on the mode of action of the chemical, and on the life cycle of the causal agent of the disease. These factors will affect timing, frequency and dosages used. Treatments can be divided into protective and eradicative types. All non-systemic or contact pesticides work best as protective treatments, not only because of their lack of mobility but also because the number of pathogen propagules to be killed is relatively small early in the development of infection. Even systemic pesticides work better at early stages of infection for the latter reason. Use commercially acceptable disease monitoring/prediction systems to determine timing when these are available.

a) Bacterial Diseases

Methods for evaluating efficacy of chemicals against bacteria causing disease are very similar in principle to those for fungal pathogens.

Consequently, bacterial diseases are covered by sections dealing with soil, seed, above-ground, greenhouse and post-harvest treatments. Specific methods have been reviewed for evaluating bactericidal sprays in controlled and field environments (17,18) and soil treatments (19). For further details consult the references.

References:

(17) Beer, S.V. and J.L. Norelli. 1986. Evaluating spray materials to control fire blight: laboratory, greenhouse and field techniques. Pp. 134-142 *in:* Hickey, K.D.(ed.), *op.cit*.

(18) Gitaitis, R.D., J.B. Jones and S.M. McCarter. 1986. Evaluation of chemical control of bacterial diseases of tomato. Pp. 205-209 *in:* K.D. Hickey (ed.), *op.cit*.

(19) Moore, L.W. 1986. Evaluating soil treatments for control of *Agrobacterium tumefaciens*. Pp. 273-276 *in:* K.D. Hickey (ed.), *op.cit*.

(b) Nematode Diseases

Methods used to evaluate efficacy of nematicides against plant diseases caused by nematodes do not differ in principle from those used to evaluate fungicides. Methods of handling and evaluating nematode populations in soil and plants have been thoroughly reviewed (20,21,22), and field procedures updated (23).

Evaluators should determine whether they are attempting to control sedentary endoparasites, migratory endoparasites, ectoparasites, or some other type, and conduct appropriate tests and follow-up evaluations. The objective is to protect the crop by single or split applications of nematicides, and to measure both the immediate effect and the duration of protection.

For foliar applications, see Section 5.6.4. For soil-borne nematode diseases see Section 5.6.2. Effectiveness of nematicides applied to soil can differ markedly, both between and within fields, in relation to variations in texture, water content, and organic matter. It is therefore essential to evaluate soils in the plot area prior to starting the test, and to use plot designs that allow for non-uniformity in nematode distribution.

References:

(20) Brown, R.H. and B.R. Kerry (ed.s). 1987. Principles and practice of nematode control in crops. Academic Press. 447 pp.

(21) Maloy, O.C. 1993. Plant Disease Control: Principles and Practice. J. Wiley and Sons Inc. New York, 346 pp.

(22) Part X. Nematicide test procedures. 1986. Pp. 281-307 *in:* K.D. Hickey (ed.), *op.cit*.

(23) Part 5. Nematicide field trials. 1992. Pp. 227-237 *in:* Muller, G. and F.J. Schwinn (eds.). Manual for field trials in plant protection. CIBA - Geigy Ltd., Basle, Switz., 271 pp.

5.6.2 Soil Treatments

Soil treatment chemicals may be divided into pre-plant and post-plant types. Pre-plant pesticides are mostly fumigants and sterilants used as biocides to pasteurize or sterilize the soil, although some are selectively nematicidal.

Pre-plant incorporation of pesticides into soils, either in dry mixes or in drenches, is sometimes used in small-scale field situations, and is used extensively in controlled environments. Because of inactivation by soil microorganisms and/or adsorption of the chemical by clay particles, it is generally considered to be an inefficient application method for field use. However, with the advent of slow-release granules, pre-plant incorporation may prove as useful in controlling seedling diseases as it is already for controlling certain insects.

Post-plant soil treatments involve pesticides applied as drenches, granulars, or sprays directed at the base of plants to prevent sudden outbreaks, e.g., of damping-off in greenhouse flats and pots, for control of root diseases, or injections into the root zone for uptake of systemics into above-ground plant parts.

(a) Soil Injection for Root Uptake

This type of pesticide application is useful for established trees, and other woody perennials, and requires systemics that are taken up via the roots and translocated to infected plant parts which may be above or below ground level. In this method, a pesticide is usually applied to the soil in the root zone. To avoid absorption by surface-layer microorganisms and organic matter, the pesticide is inserted into the sub-surface root zone via a series of holes drilled around the drip-line. It is a special case of direct soil drenching.

(b) Drenches and Granulars

Dispersal of pesticides into soil to control root and crown diseases is inefficient because most of the chemical does not reach its target. It may be adsorbed by organic matter or clay particles, or affect non-target organisms more than the pathogen. Dispersing pesticides via water drenches or cultivating granular and powder formulations into the soil can nevertheless be effective in seedbeds, hotbeds, nurseries, greenhouses, containers, and other high value crop situations. Since soil type, texture, pH, organic matter content and depth may influence the results, it is essential to describe the soil, all soil preparations, and site history carefully (27).

Pre-plant incorporation of the pesticide allows deep and thorough mixing of dry formulations. Because inoculum propagules are small, thorough incorporation or penetration of the pesticide is essential.

In-furrow sprays can place a barrier between the pathogen and the base of the stem (26). Drenches may be applied pre-emergence or post-emergence, as sprays directed at the soil surface or in irrigation water, either in sufficient volume to soak to the required depth, or watered in subsequently (23,24,25,26). Chemicals may have to be applied so that the roots do not grow out of the treated zone during the vulnerable part of their growing season (26).

Disease ratings should be taken just prior to pesticide application, and at scheduled intervals thereafter.

References:

(24) Johnson, A.W. 1986. Evaluating nematicides applied through sprinkler irrigation systems. Pp. 300-302 *in:* K.D. Hickey (ed.), *op.cit*.

(25) Johnston, S.A. and D. Hall 1986. Field evaluation of fungicides for control of Allium white rot. Pp. 194-196 *in:* K.D. Hickey (ed), *op.cit*.

(26) Kroll, T.K. 1986. Field evaluation of fungicides for control of clubroot of Crucifers. Pp. 185-186 *in:* K.D. Hickey (ed.) *op.cit*.

(27) Nesmith,W.C. and C.W. Averre. 1986. Determining & reporting soil properties in fungicide & nematicide tests. Pp. 24-28 *in:* K.D. Hickey (ed), *op.cit*.

(c) Chemigation

Pesticides may be applied mixed into irrigation water. The product label must clearly specify that the pesticide can be applied through an irrigation system. Consequently, a product must be adequately tested under irrigation before a label claim can be made. Guidelines for use of pesticides in irrigation systems have been published recently (30). Research methods have been developed for both foliar (29) and soil-borne diseases (24), including soil fumigation by chemigation when the fumigant is water soluble (28).

Chemigation is a high-volume method of applying fungicides and nematicides compared to standard low-volume applications by ground or aerial sprayers. Typically, only 10 percent of the product may stay on the leaves compared to up to 90 percent in standard applications. Improvements in control can be derived from factors such as improved distribution of chemicals, reduction of primary inoculum in debris, and less soil compaction (27). Consequently, efficacy of a product in irrigation water is usually different from its efficacy in standard sprays. Bridging data (comparative data) between chemigation and conventional application methods may be needed.

References:

(28) Adams, P.B. 1986. Soil fumigation by chemigation with Metham. Pp. 270-272 *in:* K.D. Hickey (ed.), *op. cit*.

(29) Backman, P.A. 1986. Evaluating foliar fungicides applied through irrigation systems for control of peanut diseases. Pp. 221-223 *in:* K.D. Hickey (ed.), *op. cit*.

(30) Van der Gulik, T.W. 1993. Chemigation: guidelines for British Columbia. B.C. Min. Agric. Fish. Food and Irrigation Industry Assoc. B.C., 81 pp.

(d) Fumigants

Soil fumigation utilizes chemicals that are active in the vapor phase. The essential features of all such products are that they must be thoroughly incorporated or injected to sufficient depth. The gas must fully penetrate soil particles. This requires sufficiently well-worked and aerated soil at sufficiently high temperatures. The gas must be contained in the soil for the requisite period by covering with either a gas-tight cover or by sealing the soil surface by packing or with water (21,28). The gas must then be released by removal of the cover and if necessary by aerating the soil. It may be necessary to test soil for phytotoxic residues of the fumigant before crops can be sown or planted.

Limitations to fumigant efficacy come from high soil moisture, low soil temperature, and high clay content (wet clay is almost impervious to gases), and shallow injection or incorporation. Efficacy limitations may also depend on the type and density of crop residues in the soil, initial pathogen populations, and soil porosity. If inoculum is left undisturbed deeper in the soil, it will survive treatment and attack the new crop (31). Conversely, when fumigants that act as biocides or sterilants are used, much of the soil's microfauna and microflora, the pathogen's natural antagonists, are usually killed. This creates a biological vacuum in which the first or most efficient invaders may flourish. If one or more of these is a pathogen, new and potentially more severe problems may result for subsequent crops.

It may be necessary to do follow-up evaluations of disease control and plant vigor throughout the season, and possibly in the following season. On the other hand, benefits from fumigation beyond control of the disease in question have also been found, e.g., yield increases larger than yield losses attributable to that disease. Yields obtained should be compared with yield losses estimated from disease ratings.

Reference:

(31) Moore, L.W. 1986. Evaluating soil treatments for control of *Agrobacterium tumefaciens*. Pp. 273-276 *in:* K.D. Hickey (ed.) *op. cit*.

(e) Non-target Organisms

Soil treatments of all types, especially fumigants, sterilants and biocides, may have adverse effects on non-pathogens (33). In particular, there may be marked reductions in populations of beneficial symbiotic micro-organisms such as mycorrhizae (32) and rhizobia, and on antagonists to pathogens such as *Trichoderma* spp., *Gliocladium* spp., and *Bacillus* spp. Tests on such organisms may be required and registrants are encouraged to consult the Product Manager.

References:

(32) McGraw, A.-C. and J.W. Hendrix. 1984. Host and soil fumigation effects on spore population densities of species of Endogonaceous mycorrhizal fungi. Mycologia 76: 122-131.

(33) Starr, J.L. and C.M. Kenerly, 1986. Nematicide evaluation as affected by disease complexes and nematicide effects on nontarget organisms. Pp. 305-307 *in:* K.D. Hickey (ed.) *op.cit*.

5.6.3 Seed Treatments

Seed treatments may be used to control seed-borne and soil-borne pathogens. The type of control required determines the chemicals chosen and rates of application. Pre-test and select seed lots with good germination percentage, rapid germination, and good seedling vigor for tests of soil-borne diseases (34). For seed-borne diseases, select lots with high infection percentages. Methods of seed treatment used should be reported.

Laboratory germination tests should be carried out immediately after treatment to evaluate possible phytotoxicity and at suitable intervals subsequently to support claims on how long after treatment the seed may continue to be used.

Sowing time and conditions often affect the disease level obtained and should be reported. It may be useful to monitor soil temperature and moisture as these can have a significant effect on fungicide efficacy and germination. It may be necessary to conduct seed treatment tests at cool soil temperatures to obtain enough disease pressure to demonstrate good control.

An important measure of seed treatment efficacy is stand establishment. Interim counts of emergence and of post-emergence death of seedlings are of interest in studying disease progression and evaluating efficacy. Delayed emergence may be an indicator of phytotoxicity. Before and after-winter stand counts are recommended for fall-seeded crops. Systemic disease expression often continues to appear until after heading, requiring counts later in the season. Yield data should be collected to substantiate that the effects observed on stand establishment are carried through to yield (34).

(a) Seed-borne Diseases

Seed-borne diseases are of two basic types: those where the pathogen is superficial or **surface-borne**, and those in which the pathogen has penetrated the seed coat or deeper, i.e., **internally-borne** or systemically infected.

Naturally infected seed (10-30 percent) is suitable for most diseases, but number of seeds used, plot size, or replication should be increased as the level of infection drops, or inoculated seed may be used, depending on the disease being tested. If the level in the check is too low, it may not be possible to detect treatment differences, or the results may give the erroneous impression of satisfactory control of the pathogen. If it is too high, unrealistic demands are put on the formulation and/or a dosage higher than needed may be recommended (see Section 5.3.5). Method of inoculation should be reported.

The level of acceptable disease control depends on the disease and market for the crop, e.g., 100 percent control of wheat bunt is not essential for good yield, but is required for export.

(b) Soil-borne Diseases

As with seed-borne diseases, the soil-borne diseases do not develop until the seed imbibes moisture.

(i) Damping-off

Soil-borne seed and seedling blights, collectively known as **damping-off,** may be caused by pathogens from all three major classes of fungi. Evaluators should verify that relevant pathogens are present in experimental soil or field sites.

Note that since the same soil-borne fungi may cause both pre- and post-emergence damping-off of seeds and seedlings, germination capacity (measured *in vitro*), percent germination in soil, percent emergence and percent stand establishment (measured when the seedlings are mature enough to be no longer susceptible to damping-off) are four different measures. For efficacy tests, stand establishment is the most important (35).

Where the same organisms cause both damping-off and root rot, no absolute separation between the two may be possible. Damping-off is considered to end when there is sufficient suberization of the main roots to prevent death of the seedling. Externally, the "wirestem" symptom indicates the transition from damping-off to establishment, or, if root decay persists, to root rot.

(ii) Root Rots

A second type of soil-borne disease sometimes amenable to control by seed treatment is caused by pathogens that infect at the seedling stage but are expressed as root rots of established plants, e.g., common root rot of wheat and barley. Root rots can be measured by incidence (number of affected plants), severity (proportion of roots lesioned or rotted off), plant dry weight, and seed yield.

(c) Combined Seed Treatments

Where seed treatment is used to control both seed-borne and soil-borne diseases, judicious selection can still limit the number of pesticides, i.e., one for each major class of fungi. Care has to be exercised to avoid or limit phytotoxicity since seeds are small entities to be carrying a complex load of pesticides, e.g., one or more fungicides and one insecticide and/or an inoculant. Treatments for comparison should include a reference product containing several components of the mixture to be tested (e.g., a similar formulation but lacking one of the fungicides). A slight delay in emergence may be found with combination treatment; however, this is usually compensated for by healthier plants and higher rates of establishment. It is important to evaluate combinations for any additive effects on emergence and plant growth either positive or negative (see Section 5.2.7).

(d) Coating, Pelleting and Fluid-drilling

Seed treatment chemicals may be applied in a coating material, such as methyl cellulose, which improve their retention on the seed. Components of treatment mixtures may be layered on separately in some cases. Chemicals can also be added to the drilling fluid at seeding (34,35).

References:

(34) Mathre, D.E. and E.D. Hansing. 1986. Evaluating seed-treatment fungicides. Pp. 248-251 *in:* K.D. Hickey (ed), *op.cit.*

(35) Sonoda, R.M. and S.C. Phatak. 1986. Screening fungicides for seed and seedling disease control in plug-mix and fluid-drilling plantings. Pp. 258-260 *in:* K.D. Hickey (ed.), *op.cit.*

5.6.4 Above-ground Treatments

In general, above-ground treatments aim to prevent infection by spores or other propagules by killing them on arrival at the surface of the plant, or by eradicating the pathogen at an early stage of infection.

Dispersal capability of a pathogen directly affects the size of plots used. If spores are released from the crop debris and are dispersed primarily by rain splash, then relatively small plots may be used. If, in contrast, spores are distributed by winds, not only will larger plots be needed but uninoculated plots, which may be used to determine phytotoxicity and/or efficacy of treatments, will be obtained only by covering the crop to prevent spore contact for the duration of spraying. Low-level dispersal can be restricted by using guard rows of a taller, non-susceptible species.

Researchers should measure, via disease ratings, the amount of re-inoculation, or re-start after application of fungistatic compounds, that develops following treatment. A chemical that persists and gives lasting protection against repeated natural inoculation is clearly preferable.

In general, tests should conform to current good management practices and harvesting procedures. Because of greater variability in perennial fields than within annual crops, higher numbers of replicates (six or more) may be needed.

Use of supplemental sprinkler irrigation may be needed to ensure sufficient moisture and humidity for adequate disease development (41). In the absence of irrigation, some applications should follow periods of significant wetness. Since duration of very high humidities and/or leaf wetness determine infection, rather than precipitation, these factors may be recorded throughout the test, along with air temperatures and rainfall.

Foliar applications will be most effective when applied under drying conditions at moderate temperatures, depending on the formulation. If it rains before the spray has dried, the product is likely to be washed off. At high temperatures there is more danger of phytotoxicity.

Dormant-season treatments from late fall through late winter are often useful in controlling diseases with inoculum that overwinters in bark, litter or other relatively exposed places. Such treatments are also useful in controlling diseases during spring infection periods. They may also permit use of higher dosages or more persistent chemicals because of a lower risk of phytotoxicity and the longer pre-harvest interval, provided environmental concerns are met.

(a) Annual Field Crops

This section covers cereals, oilseeds, pulses and annual forages, and includes both fall-sown winter annuals and spring-sown crops. Environmental conditions, soils and agronomic practices vary widely, and therefore require efficacy tests in a wide variety of conditions.

i) Seasonal Crop Types

Label claims must specify both the individual species and whether they are for spring or winter types. However, efficacy tests are the same for both spring and winter types, except for fall-applied treatments against snow molds. Label amendments for either spring or winter types of any given species may only require bridging data (see Section 5.2.9). A third crop type is the aquatic cereal wild rice, of which there are two species grown in Canada. This type is biologically and environmentally distinct from the other types of cereal. There are no specific use patterns for wild rice at this time.

ii) Spring Annuals

Efficacy testing methods for different annual spring crops are much the same, apart from the specifics of ensuring enough disease and characteristic expressions of some diseases that may require a distinctive measurement.

In small-grain cereals the flag leaf and the penultimate leaf provide most of the plant's photosynthates. Consequently, rating systems often concentrate on these two leaves. However, since many leaf diseases may also attack the seed heads, they should also be included in rating systems. Keys are available for rating percent area infected for leaves and heads (37). Rating the older and lower leaves may be required for corn, when control on lower leaves influences subsequent disease development, or when a change in weather prevents spread to the upper parts. In the latter case, yield is unlikely to show any differences between treatments, but rating the lower leaves may still demonstrate pesticide efficacy. Diseases that affect heads exclusively do not require leaf assessment.

Pathogens causing foliage and head diseases typically have highly dispersible spores, so that it is often difficult to prevent continued re-inoculation of treated plots. Use of taller, non-susceptible species as guard rows between plot strips has been used successfully in reducing re-inoculation. Conversely, placing rows of highly susceptible cultivars between plots can ensure uniform inoculation of the plots; however, care must be taken lest this method overwhelms the checks, giving a false result. In humid climates, repeat applications of pesticides may be needed to control re-infection of crop. In drier climates that are only occasionally

humid enough for the disease to develop, it may be necessary to provide humidity for field plots, at least during artificial inoculation, by artificial misting and/or covering for the time required for the pathogen to infect.

In broad-leaved crops, as with cereals, disease ratings are usually made on the most actively photosynthesizing foliage. Keys for scoring the percent leaf area diseased (for both cereals and broad-leaved crops) developed by James (37) are used internationally. The most useful growth stages at which to take disease ratings are the onset of flowering and the end of flowering, as these may correlate well with damage to vegetative growth and reproductive growth respectively (34,36); however, the most useful stages for ratings also depend on disease type and development. The last disease assessment is usually at the onset of senescence. Assessments at later stages are required only where post-senescence disease development affects quality, e.g., microbial discolorations reduce malting quality of barley.

iii) Winter Annuals

Winter annuals (fall rye, winter wheat, winter barley) are sown in the fall and are subject to damage by many pathogens. Summer diseases are controlled as for spring annuals. Winter annuals are also subject to several different snowmolds which may occur in mixtures. Efficacy claims must indicate which pathogens are controlled. Chemical protection is required for up to six months in some regions of Canada. Some of the fungicides used may have beneficial effects on soil-, residue- or stubble-borne spring diseases as well.

The most useful parameters for measuring efficacy against snowmolds are, in descending order, number of seed stems or heads per unit area, number of plants established (counted approximately four weeks after resumption of growth in the spring) as a percent of the number at the time of fall treatment, and yield. Yield is an indirect measure of treatment effect, but is the best indicator of economic benefit.

References:

(36) Frank, J.A. & H. Cole. 1988. Field evaluation for control of foliar diseases on small grains. Pp. 224-225 *in:* K.D. Hickey (ed.), *op. cit.*

(37) James, W.C. 1971. An illustrated series of assessment keys for plant diseases, their preparation and usage. Can. Plant Dis. Surv.51:39-65.

(38) Nesmith, W.C. 1986. Evaluating fungicides for control of foliar diseases of tobacco. Pp. 226-230 *in:* K.D. Hickey (ed.), *op. cit.*

(b) Perennial Field Crops

This section applies to all types of field-grown grasses, legumes and other herbaceous perennial crops. A grass or legume may be grown for forage in pure stands for forage or seed, in defined mixtures, with one or more other species for pastures, or in mixtures for range and brush lands, and singly or in various mixtures for reclamation and soil stabilization. Seed fields may be grazed following harvest. Preliminary screening of fungicides and nematicides to establish dosage ranges and thresholds of phytoxicity may be done in the greenhouse, as with other crops (38).

i) Foliage, Stem and Head Diseases

Foliage, stem and head diseases of perennials may be controlled chemically. Particular attention must be paid to stages of infection and disease development for good results. Sources of inoculum include plant debris and stubble from the previous crop. Scattering hay or debris from a heavily infected field of the previous year is an effective way to inoculate plots for test purposes. This is best done in the fall so that immature sporophores may mature naturally by the following spring, and it is less weather-dependent than spraying relatively ephemeral conidia.

In perennial fields, there are usually several diseases present. Even when the test site has been selected for dominance of the disease being treated, it is necessary to evaluate all the diseases present (33,39,41). With multiple-cutting crops, each forage harvest or cutting stage should be rated as well as harvested.

It is important to follow field practices and harvesting procedures that favorably affect quality factors and price (41).

ii) Snowmolds

Perennials are attacked by the same snowmolds that damage winter cereals. In complexes of snowmolds, the relative dominance of the different pathogens is likely to change with age of the stand, depth of snow cover, and agronomic practices.

Consequently, fungicide mixtures usually prove more effective than single compounds. Methods for assessing efficacy are the same as for cereals, except for multi-season measurements and measures of forage quality and yield.

References:

(39) Orr, C.C. and C.M. Heald. 1986. Plant responses in the evaluation of nematode control agents. Pp. 297-299 *in:* K.D. Hickey (ed.) *op. cit.*

(40) Sanders, P. and H. Cole. 1986. *In vivo* fungicide screening on greenhouse-grown turfgrasses. Pp. 110-111 *in:* K.D. Hickey (ed.) *op. cit.*

(41) Stuteville, D.L. 1986. Methods for field evaluation of fungicides for control of foliar diseases of alfalfa. Pp. 210-211 *in:* K.D. Hickey (ed.) *op. cit.*

(c) Turf

Turfgrass diseases may be controlled with fungicides, nematicides or pre-plant fumigants (see Section 5.6.2c). Turf stands are very uniform compared to other crops, including the same grasses when grown for seed or forage, which allows use of small plots, e.g., $1m^2$, provided inoculum is also uniform.

The modern golf green is a relatively artificial form of turf grown mainly on sand. It is also managed much more intensively than other types of turf. Such turf is more sensitive to overdosing, quicker to show phytotoxicity and quicker to die out than turf grown on soil. Consequently, more precision and care is needed in applying pesticides, and more frequent observations and attention to signs of toxicity are required than for other turfs. Moreover, pathogens in such conditions are more liable to produce resistant strains unless fungicides of different modes of action are used.

Other types of amenity turfs may be grouped together for efficacy testing, although management practices and underlying soil type may have marked effects on disease occurrence and control. Turf damage and control may be evaluated by estimating the percent area of the plot damaged, or by mapping and measuring damage, or quality and color, using a planimeter, and rating the severity of damage. Taking soil temperatures may be useful in evaluating root diseases such as take-all patch, e.g., at 5 cm depth, at 2 p.m. The ability of turf to regenerate from deep tillers, i.e., its recovery rate, is also affected by treatments and should be assessed at predetermined intervals. Time required for full recovery should also be determined.

In climates where freeze-up of the ground occurs, it is essential that treatments be applied beforehand. Tests should also include some treatments applied as soon as there is frost at night because some
so-called snowmolds become active once the turf starts to become dormant, and cause most of their damage prior to freeze-up. Timing, duration, compaction and depth of snow have marked effects on snowmold damage as well as temperature at the groundline. All of these variables should be monitored (42).

Complexes of diseases may build up over time in turfs, some acting synergistically, but others acting at different seasonal stages. The effect of treatments on other diseases present should be evaluated. These treatments may be beneficial or, by controlling a certain disease, create a biological vacuum that allows another, perhaps insignificant, pathogen to cause considerable damage (42). Use disease-prediction systems when available.

References:

(42) Smiley, R.W., P.H. Dernoeden and B.B. Clarke. 1992 (2nd.ed.). Pp. 80-82 *in:* Compendium of turfgrass diseases. APS Press, Amer. Phytopath. Soc., 98 pp.

(43) Smith, J.D. 1987. Winter-hardiness and overwintering diseases of amenity turfgrasses with special reference to the Canadian prairies. Agric. Can. Res. Br., Tech. Bul. 1987-12E, 193 pp.

(d) Vegetables and Herbaceous Fruits

Field trials for vegetables and herbaceous fruits are laid out according to the same principles as for other field crops. Since individual vegetable plants are generally larger than those of cereals or grasses, plot size may be calculated on some minimum number of plants. This, in turn, may depend on the number of plants required for a sample, and the number of samples that have to be taken during the season. As plant size increases, the number per sample or per plot decreases. For larger vegetables, e.g., potatoes, cucurbits, or melons, minimum sample size is 5-10 plants leading to a minimum of 20-40 plants per plot. For smaller species, e.g., carrots, onions, or strawberries, a minimum plot size may be 5m of row. Where applicable, both fresh market and processing cultivars should be tested.

Plots may be separated by untreated guard rows to minimize spray drift, unless there is a risk that the disease may build up so much that it overwhelms the treated rows. Single-row plots can be used where there is little risk of spore or spray drift, whereas square plots astride several rows are preferable when either spores or sprays may drift. When plots of three or more rows are used, the outside rows serve as treated guards and the inner rows, which are free of edge effects, are used for sampling, rating and harvesting.

Where growers follow weather or weather-prediction systems in setting their spray schedules, some of the efficacy tests should be done similarly.

Disease measurement should include not only direct measurements of symptoms, but also a quality assessment of factors such as poor size, blemishes, rot and other defects (44). Loss of quality may be more important economically than loss of quantity. Unless otherwise specifically indicated, yield means commercially marketable yield. However, gross yield and yield of each commercial grade may

also be taken. Each harvest should be graded, rated and stored separately.

For vegetables and herbaceous fruits, both efficacy and toxicity evaluation may continue after harvest, as both disease and treatments can affect storage and even taste. Disease assessments of vegetables stored under commercial or comparable conditions should be made at appropriate intervals.

Reference:

(44) Howard, R.J., J.A. Garland and W.L. Seaman (eds.). 1994. Diseases and pests of vegetable crops in Canada. Can. Phytopath. Soc. and Entomal. Soc. Canada, Ottawa, Ont. 554 pp.

(e) Orchards, Bush and Vine Fruits

Orchard trials with tree, bush and vine fruits are laid out according to the same principles as other field trials. The large size of established plants may reduce the number of plants per plot to as little as one for well-established trees. To avoid problems of scale and replication with larger trees, it is feasible to do some efficacy testing of fungicides on potted trees and shrubs, 1-10 years old, in the greenhouse, or using detached blossoms (36,37). These techniques are also suitable for preliminary efficacy testing to establish dose rates or concentrations.

In orchards, good commercial management practices should be used, including annual pruning, and pruning before setting up a trial, which help to establish and maintain open canopies that permit thorough spray penetration, stimulate new wood production in the lower part of the plant, and help to develop a sound framework for long-term production. Judicious commercial pruning makes it possible to maintain twice the usual number of trees or shrubs in the early years of a test site, and allows for earlier and more intensive testing by doubling production and maintaining a more favorable microclimate for disease development (46). It also increases the density and effectiveness of buffer rows.

Weather-based prediction systems have been developed for some orchard diseases, e.g., apple scab (47). To use these effectively, it is essential to know when infection periods occur, the length of the incubation period, and the severity of subsequent infections. Considerable delays in symptom development may occur with consequent delays in evaluation, e.g., until the following spring for bud infection, whereas blossom infections may develop in as little as two days. Where recommended spray schedules have been developed, consult a local pest management specialist about their use.

Initial field testing can be done with handgun sprayers and single- or half-tree or vine replicates. However, commercial orchards are generally treated using some form of air-blast sprayer. Custom versions may have to be made for efficacy testing

(47). It has been recommended that air-blast plots should consist of a minimum of five trees times five replicates. Others use one to three tree plots replicated four times. The size of experimental orchard plots depends in part on the size of the trees. Buffer rows are required between treated rows because air-blast sprayers will distribute sprays over a considerable distance even on calm days (47).

Since shrubs, vines, and herbaceous perennials are grown in rows, the unit for testing is a few metres of row, which will be determined by the space occupied by a certain minimum number of plants. In others, the row may be so grown together that individual plants can no longer be distinguished which may limit evaluation of systemic pesticides.

It may be necessary to evaluate efficacy against root and crown diseases on both rootstocks alone and on grafted commercial cultivars. As with all diseases of woody parts of trees and shrubs, evaluation of treatment effects may take two or more years.

Bactericides are usually tested separately from fungicides, although a fungicide and/or an insecticide may be used as a background treatment in order to isolate the bacterial disease. Preliminary screening of test materials using seedlings or immature fruit is strongly recommended, in order to minimize the number of times and locations requiring inoculation with a virulent disease (47). When treatments are applied to blossoms, inspections and ratings of both the disease and phytotoxicity are often done at two-day intervals (46).

Disease ratings can be taken on as few as 20 branch terminals per plot or per tree provided they are selected at random. This allows for a detailed evaluation of each terminal. Numbers of fruit, leaves and nodes (where fruit or leaf drop is a sign of disease) can be counted and several ratings can be made at different stages of the season. Ratings should allow for the sizes of the twigs, branches or trunks affected by disease, and type of product used (47).

Fruit yield and quality factors should be evaluated. Commercial yield is the principal factor incorporating visual quality factors. For full information on fungicide performance, measure gross yield, yield in each commercial class, and rate quality factors. Quality factors vary with intended use and differ for fresh fruit juice, processing, drying and, in the case of grapes, wine in which effects on fermentation yeasts, aging and taste development may occur.

References:

(45) Jones, A.L. and G.R. Ehret. 1986. Field evaluation of fungicides for control of diseases on tart cherries. Pp. 120-124 *in:* K.D. Hickey (Ed.) op. cit.

(46) Szkolnik, M. 1986. Investigating physical modes of action of tree fruit fungicides. Pp. 98-101 *in:* K.D. Hickey(ed.)

(47) Szkolnik, M. and K.D. Hickey. 1986. Testing chemical sprays on blossoms of deciduous fruit trees. Pp. 112-115 *in:* K.D. Hickey (ed.) *op. cit.*

(f) Nurseries and Short-lived Plantations

Nurseries, forest tree nurseries, caliper tree and Christmas tree plantations, and outdoor container stock may require production of blemish-free plants in predetermined size ranges. Pre-plant fumigants, drenches and granulars (Section 5.6.2) are widely used to eliminate initial soil-borne diseases and nematodes (39). Seed treatments (Section 5.6.3) are used where soil is not treated. Principles for efficacy testing on nursery crops are very similar to those for vegetables, herbaceous plants and orchard crops.

Phytotoxicity is of great concern in nursery crops because they are judged and marketed on their appearance (48). Their health and vigor, which are mutually dependent, are also at a premium so as to optimize transplant survival. Latent infections that cause disease only after transplanting are an industry-wide problem that requires production of disease-free plants. The objective, therefore, is always 100 percent efficacy; however, the repeated pesticide applications that are required may lead to development of pesticide-resistant strains of pathogens.

Test procedures should not only allow for evaluation of efficacy, but also of phytotoxicity, vigor, latent infections, and the presence of resistant strains. Latent infections may be caused by resistant pathogen strains whose activity is suppressed by the routine prophylactic measures, but which are able to develop when controls are relaxed after transplanting.

To evaluate phytotoxicity, higher dosage rates should be included. For foliar diseases, tests should include two to four applications per month for three to six months. Ratings should be made to evaluate both efficacy and phytotoxicity. The most appropriate rating may not be apparent until after the trial is completed. Photographs should be used to document qualitative scales to assist reproducibility of tests (48).

References:

(48) Chase, A.R. and D.D. Brunk. 1986. Evaluating fungicides for control of foliar diseases of foliage plants. Pp. 240-243 *in:* K.D. Hickey (ed.) *op. cit.*

(49) Sutherland, J.R., G.W. Shrimpton and R.N. Sturrock. 1989. Diseases and insects in British Columbia forest seedling nurseries. FRDA Rep. 065, Can./BC Econ. Reg. Dev. Agrmt. and B.C. Min. For., 85 pp.

(g) Shelterbelts, Landscapes and Parks

Trees and shrubs in shelterbelts, landscapes, parks and other public-use areas are subject to a variety of diseases, some of which can be controlled with commercially registered fungicides or bactericides. Similarly, ornamental herbaceous perennials and annuals may require chemical control of their diseases.

Testing may not be acceptable in public-use areas, however, and should be carried out in areas in which access can be controlled. The same species and cultivars grown in public-use areas should be used in the tests.

Efficacy testing methods for ornamentals in public-use areas are similar to those for orchards, nurseries and vegetables. The large size of some trees poses practical problems in application, and public use raises safety issues, so that testing of such crop/disease combinations is most readily done in nurseries or plantations.

(h) Tree Injection

Injection of pesticides is used to control diseases in individual trees of high economic or aesthetic value. Strictly, **injection** refers to the introduction of fluids via a pressurized apparatus, either low pressure (syringes, garden sprayer tanks, gravity bags) or high pressure (N, CO_2, or compressed-air tanks). Passive uptake of fluids through various types of wounds is termed **infusion**.

Problems arise from a lack of standard methodologies. All injection methods require wounding the tree, and the tree responds to wounding by plugging its vessels, sometimes within hours, thereby preventing further uptake. Furthermore, intake and translocation are different for each pesticide, and each may be translocated differently from the dyes often used to monitor internal movement (50).

The most uniform distribution of compounds within a tree comes from infusion or injection of lateral roots (cf. Section 5.6.2(a)). The higher up the tree that injection takes place, the more unpredictable the uptake becomes (47).

Efficacy testing is the same as for other trees, except for method of application. If pesticides are applied at early stages of infection, remission of foliar symptoms is

possible and should be documented. The least expensive method is with a visual rating method. Standard photographs should be used to illustrate the different rating grades, and to provide year-to-year consistency and reliability of assessments (51). Where fruit trees are treated, the appearance, taste and marketable yield of the fruit should be recorded. Tree vigor may be evaluated by measuring elongation of shoots, trunk diameter, height, and increase in branching (51).

References:

(50) Lacy, G.H. 1986. Evaluating chemicals for tree infusion or injection to control diseases caused by mycoplasmalike organisms. Pp. 266-269 *in:* K.D. Hickey (ed.) *op. cit*.

(51) Stipes, R.J. and R.J. Campana. 1986. Introducing and evaluating liquid fungicides in elm trees for the control of Dutch Elm Disease. Pp. 261-265 *in:* K.D. Hickey (ed.) *op.cit*.

(i) Ranges and Forests

At present, there are few applications for chemical control of plant diseases in range or forest lands. See Part 3: Herbicides, for methods appropriate to such lands.

(j) Domestic Areas

Gardens, yards, housescapes and other private-use areas may be treated only with pesticides having DOMESTIC (DOM) registration. The differences between DOMESTIC and COMMERCIAL (COM) registrations relate to safety and residue concerns rather than to efficacy or phytotoxicity. Methods of efficacy testing, therefore, are the same as in the sections above on vegetables, fruits, and ornamentals.

5.6.5 Greenhouse and Other Enclosed-Space Treatments

(a) Greenhouse, Tunnels and Row Covers

This section deals with greenhouse-grown crops and, by extension, crops grown under cold-frames, hot caps, row tunnels and other types of covers. Use of greenhouses and growth cabinets as research tools in evaluating pesticide efficacy on field crops is included under those crops. Treatment of greenhouse soils and mixes is similar in principle to field soil treatment (Section 5.6.2), except that the smaller scale permits greater precision and uniformity. Greenhouses may also be used for precision studies on fungicide modes of action, phases of activity, and redistribution (46).

The greenhouse environment, however, is far from uniform. Light and temperature gradients often occur from the glass walls to the centre of the house. These gradients can readily be measured. Care must be taken to provide adequate replication and randomization of pots, trays or other container units. For soil-borne

diseases, keep the growth units separate and do not use tray-benches with common sub-unit irrigation. Likewise, if automated irrigation is used, its backflow prevention must be accident-proof for soil treatments. To ensure that foliar diseases develop, use overhead irrigation or misting and close spacing of plants in order to develop a closed canopy, thus creating a humid micro-environment that favors disease development.

In tests of fungicides in fluids applied as sprays or drenches, the principles of efficacy testing are much the same as for field trials. Because of the favorable growing environment for protected crops, fungicide applications may be at frequent intervals. Some diseases, however, may require three or more months for symptom expression even in the greenhouse and evaluations must allow for this period (46).

Frequent and regular greenhouse fungicide schedules may suppress disease symptoms for as long as the schedule is maintained. However, when an infected plant reaches the marketplace, disease can develop. Evaluation protocols should therefore include relatively long-term follow-up measurements of disease.

i) Fumigation

Since the greenhouse is an enclosed space, it is frequently sanitized aerially by fumigation chemicals. These may include fungicides active in the vapor phase (48,52). Where only one room is available, plants may be treated for test purposes in canopies placed over them for the treatment period, typically
6-48 hours, or placed in a treatment chamber and then returned to the bench. Any greenhouse that can be adequately sealed during fumigation can be used to test the volatile activity of fungicides (52).

Reference:

(52) Coyier, D.L. 1986. Testing procedures to determine the volatile activity of fungicides for control of powdery mildews in the greenhouse. Pp. 102-104 *in:* K.D. Hickey (ed.) *op. cit.*

(b) Interiorscapes

One of the fastest-growing areas of ornamental production is plants for homes, office buildings, and shopping malls where the environment may be substantially different from production greenhouses and nurseries. The far-ranging and rapid transport of container plants throughout the world potentially creates an equally far-ranging set of disease problems, and greatly increases the demand for pathogen-free plants. Latent infections are increasingly serious for both the end user and border officials (53,54).

Efficacy testing against diseases of interiorscape plants while in production in greenhouses or nurseries is the same as for other greenhouse and nursery plants. Registration for pesticides in interiorscapes is primarily a matter of meeting safety requirements to get a DOMESTIC (DOM) classification for chemicals previously registered in COMMERCIAL (COM) use. The actual efficacy testing can be done in greenhouses unless it is shown that this produces a different result than in the interiorscape.

References:

(53) Chase, A.R. 1986. Compendium of ornamental foliage plant diseases. A.P.S. Press, St. Paul, Minn. 114 pp.

(54) Howard, R.J. and A. Buonassissi. 1992. Diseases of interiorscape plants. Ch. 13, 26pp. *in:* Guidelines for the control of plant diseases in Western Canada, West. Comm. Plant Diseases.

(c) Mushroom Houses

i) Commercial mushrooms (*Agaricus bisporus*) are subject to diseases caused by fungi, bacteria, nematodes and viruses, and can also experience significant problems from competitive weed molds. Some of these diseases can be chemically managed.

Chemicals may be used to treat the spawn (the vegetative inoculum), the growing materials or the empty growing facilities. The spawn grains can be coated with a chemical to protect this cereal grain from being used as a food source by pathogens or competitive fungi. The growing materials or mushrooms could be treated with irrigation drenches or dusts at any time during the crop. The empty facilities (or parts thereof) may be disinfected with steam or sanitizers.

Efficacy tests may require the simultaneous treatment of several production rooms. The small number of plots (production units) may restrict the statistical inferences, although this may be partly offset by suitable subsampling techniques within each production unit. Subsampling may also be used to evaluate the disease and efficacy gradients within any production unit. Care should be taken to ensure uniform distribution of the test material in the production room.

Efficacy ratings should include not only the quantity of marketable mushrooms but the quality of these as well. Efficacy ratings may also include the count of non-marketable mushrooms.

Pesticide tolerance of the crop is a critical component of a mushroom pesticide evaluation. Rate, timing of application and mushroom strain (cultivar) are important aspects in a thorough assessment. An appropriate statistical design, taking into account the three-dimensional arrangement of production, and discrete applications to these subsample units within the production room can adequately assess the effect on a crop.

Crop tolerance ratings should include not only the effect on crop yield, the quality of the mushrooms harvested but also the timing to harvest.

ii) **Other mushroom species** are grown under conditions particular to the species. Efficacy and crop tolerance tests must be carried out separately for each species under the conditions appropriate for that species before claims can be established or put on the label.

References:

(55) Rinker, D.L. 1993. Commercial mushroom production. Ont. Min. Agric. Food and Rural Affairs, Publ. 350.

(56) Rinker, D.L. and P.J. Wuest. 1994. Mushrooms. Pp. 363-379 *in:* Howard, R.J., J.A. Garland and W.L. Seaman (eds.), Diseases and pests of vegetable crops in Canada. Can. Phytopath. Soc. and Entomol. Soc. Can., 554 pp.

5.6.6 Postharvest Treatments

(a) General

Postharvest diseases may be divided into two broad groups, those that develop from infections that occur in the field prior to harvest, and those which develop from infections or wounds permitting subsequent infections generated during harvest or pre-storage handling.

In general, any treatment that helps to produce disease- and blemish-free plants and produce can be considered part of a program to control postharvest problems caused by preharvest infections. Efficacy testing for such pesticides is already covered above under various types of field testing. Nevertheless, there are specific treatments aimed at controlling postharvest diseases as early as pre-planting treatment, e.g., bulb treatment for neck rot of onions. There are sprays applied at the end of blossoming against blossom-end rots expressed either in the field or in storage (56). Most treatments against preharvest infections, however, are applied in the preharvest period. All of these require evaluation in/and following storage.

It is important to understand what causes different postharvest diseases. Pathogens causing preharvest infections, whether trimmable or quiescent, tend to be host-

specific. Those that infect harvesting and handling wounds tend to have broad host ranges.

The major sources of variability in efficacy testing on produce are differences in maturity or curing of individual fruits or vegetables, and lack of uniformity in inoculation and pesticide application. Therefore, use 50-100 fruits or vegetables per treatment, a minimum of 10 per subsample, and a minimum of four replicates. Units of replication vary with the crop, the methods by which it is handled commercially, the type of treatment being applied, and the stage of testing. Final testing for registration applications should be on commercial-size lots (59).

Evaluations should be made at the time of treatment, at two or more intervals in storage, at the time of removal from storage, and in the marketplace. Some diseases are quiescent in cold storage, and only become a problem during shipping or marketing. Consequently, evaluation of the test should continue through a complete growing cycle, and perhaps an additional storage and growth cycle to determine the residual activity and phytotoxicity of the pesticide.

The pesticide should be tested with each type of equipment (hydro-coolers, dump tanks, tank washers, spray washers, brush cleaners, etc.) named in the label claim.

(b) Fruits and Vegetables

(i) Preharvest treatments

Preharvest treatments aim to minimize quiescent infections and destroy inoculum that can be carried with the crop and distributed during postharvest handling. These are mostly sprays applied and evaluated according to the same criteria indicated above for field crops, except that evaluation continues through storage. To prevent cross-contamination, wear disposable gloves for harvesting and handling, changing gloves after each treatment. Storage should be at temperatures, humidities and atmospheres appropriate for each crop. Preharvest treatments are used primarily where postharvest treatment is not practical, especially when produce is sold at the farm gate or local markets.

(ii) Postharvest treatments

Wet, or non-volatile, fungicides and bactericides may be applied in hydrocooling water, dump tanks, dips, drenches, sprays and waxes. Volatile pesticides may be applied as fumigants, vapors, fogs or smokes. Each type of application requires separate testing to establish label claims.

Select produce that has not been treated in the field, and measure its maturity before, during and after treatment and storage, as maturity usually

affects resistance to the pathogens.

It is important to simulate commercial conditions as closely as possible, including number, turbulence and duration of rinsings, brushing or other handling, air drying systems and durations.

Liquid treatments must wet thoroughly the often waxy surfaces of produce, for which reason a suitable surfactant is usually added (58). In pesticide combinations used to combat pesticide-resistant strains, each component must be tested separately, as well as together, and each treatment including the check must contain any surfactant or other additives used. Observe for either enhanced or diminished control with pesticide combinations, and for interactions with surfactants. In simulating commercial conditions, note the increase in spore levels resulting from recycling the water.

Testing must be done at appropriate air and fluid temperatures. Hot fungicides are more effective than cool ones, and their use should lead to reduced dosages. Otherwise, there is a risk of higher phytotoxicity (57,58). While greater penetration compensates for reduced dose, it can lead to increased residues. Heat tolerances of individual crops must be closely observed. In general, water used in cleaning, treating and grading operations for refrigerated produce is at least 5°C warmer than the incoming produce to prevent a pressure differential that would force water and inoculum into stem scars, lenticals and wounds. Whereas freshly harvested produce is usually hydrocooled in chilled water to remove field heat.

iii) Solvent waxes

Solvent waxes are applied at low volume and dry fast, allowing less time, less coverage and less penetration than water applications. Wax treatments are less effective at a given dose, and are usually not tested until water suspensions or solutions have been proven effective (57,58).

iv) Fumigants

Fumigants are tested in closed compartments, usually storage compartments. Care must be taken to ensure good spacing and adequate vapor mobility, and to reproduce commercial conditions (57,58).

v) Micro-fumigation

Micro-fumigation utilizes impregnated pads, foams or wraps to provide control in shipping cartons, both in storage and in shipment (57,58).

vi) Root and Tuber Crops

Root and tuber crops are cured after lifting, cleaning, trimming and air drying, prior to treatment and placing in storage. Care must be taken in efficacy testing to reproduce commercial curing since this greatly affects the healing of wounds and trimming cuts. Curing procedures are specific for each crop. Treatments need to take into account the end use of the crop, e.g., potatoes for seed or for the table, as this affects dosages, duration of required protection, storage conditions, pre-marketing intervals and permissible residues (58).

(c) Flowers and Foliage

For floricultural crops, testing during growth is conducted according to the same principles as other greenhouse crops, although acceptable levels of phytotoxicity and visible blemishes and spray deposits are more restrictive.

Storage problems for cut flowers and foliage are largely avoided by speed of delivery and cool temporary storage. For potted plants, latent or quiescent infections are increasingly serious as partially resistant strains become more frequent. However, these are a variant of greenhouse crops or interiorscape plants and testing is the same (Section 5.6.5).

(d) Seedlings, Bulbs and Other Propagative Units

Tests should closely simulate, or be done under, commercial conditions to be useful. Mold on forest seedlings and other fall-dug, fall-stored woody plants (ornamentals, fruit trees, shrubs) in storage has become a major problem because of the increasing length of storage periods, whether for bare-root or container-grown stocks (59). Increased production of container-grown stocks, which may contain incipient molds, is a contributing factor to this problem.

Tests to control storage rots of ornamental bulbs, corms, tubers, root segments, etc., should be conducted with 4-6 replicates and 50-60 bulbs per replicate to allow for variability between bulbs, inoculation and disease development (58). For efficacy testing, if natural infection is used, select apparently healthy bulbs from heavily diseased lots. For inoculation and for phytotoxicity tests, select healthy bulbs from disease-free lots. The principal disease ratings are taken at the end of storage, at the time of planting and at establishment about 30 days later. However, full testing requires evaluation through a complete growth cycle, a second storage period and the subsequent establishment period. Disease and phytotoxicity should be measured at both the flowering stage and at season-end bulb harvest (58).

References:

(57) Eckert, J.W. and G.E. Brown. 1986. Evaluation of postharvest fungicide treatments for citrus fruits. Pp. 92-97 *in:* K.D. Hickey (ed.) *op. cit*.

(58) Eckert, J.W. and J.M. Ogawa. 1988. The chemical control of postharvest diseases: deciduous fruits, berries, vegetables and root/tuber crops. Ann. Rev. Phytopath. 26: 433-469.

(59) Meheriuk, M. and W.J. McPhee. 1984. Postharvest handling of pome fruits, soft fruits, and grapes. Agric. Can., Publ. 1768E, 50pp.

(60) Rosenberger, D.A., R.A. Spotts, W.S. Conway and K.S. Yoder. 1986. Evaluating fungicides for control of postharvest decay of pome fruits. Pp. 88-91 *in:* K.D. Hickey (ed.) *op.cit*.

Table 1 Summary of Field Data Required

Information Required	Proposed Use Pattern								
	Soil	**Seed**	**Foliar**					**Green House**	**Post Harvest**
			Field Crops	**Turf**	**Veg/ Fruit**	**Orchard**	**Forest**		
Confirmation of Pathogen	I or A	I or A	N or A	N or A	N or A	N or A	N or A	I or A	I or A
Disease Assessment	E and S	E and S	S	S	S	S	S	E and S	S
Plant G.S. *	/	/	/		/	/	/	/	
Treatment Application Dates	/	/	/	/	/	/	/	/	/
Phytotoxicity	/	/	/	/	/	/	/	/	
Yield	/	**	/		/	/	/	/ except ornamentals	
Quality			MY		MY	MY		MY	MY

I - isolation of pathogen before or after treatment
A - artificial inoculation (not necessary to re-isolate)
N - natural inoculum, estimated by disease incidence in untreated plants
E - emergence counts
S - disease symptom incidence and severity
MY - marketable yield, other quality factors
* - plant growth stage at time of treatment application and disease assessment
** - may be required, e.g., for smut.