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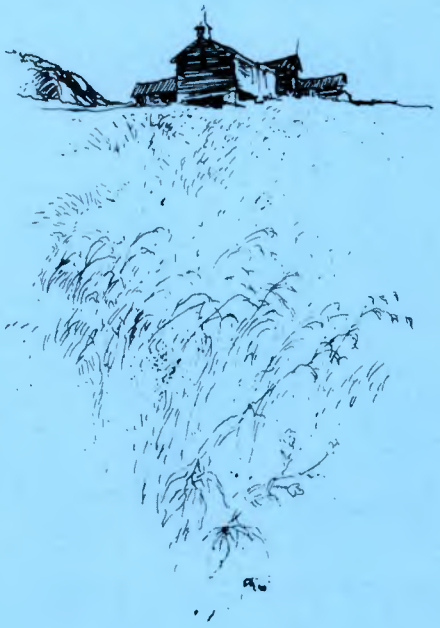

Norwegian Agricultural Advisory Centre, Ås, Norway

NORWEGIAN JOURNAL OF AGRICULTURAL SCIENCES

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CORRESPONDENCE

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NORDIC GUIDELINE FOR THE BIOLOGICAL EVALUATION OF PESTICIDES

This guideline is made by a working group under the Nordic Committee for Biological Evaluation of Pesticides.

The aim is to provide a general scheme for testing of pesticides in the Nordic countries and to improve the cooperation concerning biological evaluation and to make the testing of pesticides standardized and efficient.

The guideline is based on EPPO guideline and national guideline for biological evaluation of pesticides and contains a short description of the biology of noxious organisms and how to establish, manage, evaluate and report plant protection trials.

The work has been financed by the Nordic Council.

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NORDIC GUIDELINE NO. 1

1. POWDERY MILDEW OF CUCURBITS

1.1. Pathogens

Powdery mildew of cucurbits, caused by *Sphaerotheca fuliginea* (Schlecht. ex. Fr.) Poll and occasionally by *Erysiphe cichoracearum* (DC ex. Mérat em. Salmon).

Plants in a glasshouse can be infected by either *S. fuliginea* or *E. cichoracearum.*

S. fuliginea have egg-shaped conidia with inclusions of irregular fibrosin bodies. Germ tubes are usually branched. Conidia of *E. cichoracearum* are more angular; they have inclusions and germ tubes are unbranched.

1.2. Hosts

Cucumber *(Cucumis sativus),* melon *(Cucumis melo),* pumpkin *(Cucurbita pepo).*

1.3. Symptoms

Symptoms are the same on all three species. Normally only the conidial stages of the organisms are seen. Powdery mildew develops small white spots which quickly expand and may cover upper surfaces of leaves completely. Other parts of the plant are more sparsely colonized.

1.4. Epidemiology

The pathogens overwinter in conidial stadium in greenhouses and surrounding areas. The perfect stadia are of minor importance (cleistothecia are seldom seen) for the survival of the fungi.

1.5. Possibilities for Misidentification

None.

2. EXPERIMENTAL CONDITIONS

2.1. Selection of Crop and Variety

Normally, a susceptible cucumber cultivar; one cultivar is used for the whole test.

2.2. Environmental Conditions

The trial should be carried out in a glasshouse, attention being paid to maintaining homogeneous culture conditions. Separate glasshouses or separate glasshouse compartments must be used for each treatment if products with a high vapour pressure, fumigants, areosols or fogs are being tested.

2.3. Disposition and Size of Plots

Components: product(s) to be tested, reference product(s) and untreated control, arranged in a randomized complete block design alt. systematic block design. Plot size (net); at least 5–10 plants. Replicates, normally 4. Plots should be separated by buffer plants and buffer rows of a resistant cultivar. The minimum plot size given is appropriate for high and medium volume spray applications. If products with high vapour pressure, fumigants, aerosols or fogs are to be tested, separate glasshouse compartments must be used. In such cases, the trial may be carried out without replicates.

2.4. Inoculation
In order to ensure infection, the crop may be inoculated (e.g. by attaching 1 cm discs from a heavily infected leaf, for 24 h, to the leaves to be infected). The method used should be recorded.

3. APPLICATION OF TREATMENTS

3.1. Product(s) to be Tested
According to the trial description.

3.2. Reference Product(s)
Registered product(s) having widely proved satisfactory in practice. Formulation type and mode of action should be as close as possible to those of the product(s) to be tested.

3.3. Mode of Application

3.3.1. Type of Equipment Used
Application with currently used equipment. The system used should provide an even distribution of product on all plants in the plot. Give information on the type of equipment and operating conditions.

3.3.2. Time and Frequency of Application
According to the directions of use of the applicant. If no recommendations are made, the first application should take place when the infection is first noticed. The product will normally be reapplied at 7-day intervals, at least three times. Alternative number and length of intervals should be used if requested. The number of applications and the date of each, as well as the state of development of the plants, should be recorded.

3.3.3. Doses and Volumes
The product should normally be applied at the dosage recommended by the applicant.

Plants should be sprayed until total wetness (normally calculated at 250 – 350 ml solution on each plant). Equipment for low pressure spraying should be used in order to achieve maximal wetness. Data on concentration (%) and volume (litre/ha) should be given. In some cases the dosage may be given as a concentration combined with a volume commensurate with the state of the crop.

3.3.4. Data on Chemicals Used Against Other Pests, Diseases or Weeds
If other chemicals have to be applied, provision must be made to ensure interference is minimal. These chemicals have to be applied uniformally on all plots and, normally, should be applied separately from the product(s) being tested and the reference product(s).

If biological control of insects or pathogens is being carried out, care should be taken that organisms are uniformally spread on all plots. Precise data on these applications must be given (time of treatment, stage of development of the plant and pesticide or organism used).

4. MODE OF ASSESSMENT, RECORDING AND MEASUREMENTS

4.1. Meteorological Data
Record temperature and relative humidity continuously during the entire test period.

4.2. Assessment of Effect of Fungicide(s) on Powdery Mildew of Cucurbits

4.2.1 Time and Frequency

The first assessment: immediately before treatment.

Intermediate assessment: at least three intermediate assessments should be made, timed according to disease development (generally just before the next treatment).

Final assessment: 7–14 days after the treatment. Plants treated with eradicant fungicide should have their leaves marked and infection level repeatedly controlled.

Date of each record should be noted as well as state of development of the plant.

4.2.2. Type

Assess the percentage leaf area affected on upper surfaces on the 10 oldest green leaves of at least five plants per plot. A scale such as the following may be used:

Grade	*% leaf area infected*
1	0
2	0 – 1
3	2 – 5
4	6 – 20
5	21 – 40
6	40

If infection is light, it may be useful to count numbers of mildew spots per plant.

Brown mycelium is supposed to be dead and should not be counted in the assessment.

Calculation of infection degree (P) according to Townsend & Heuberger:

$$P = \frac{n \times (v - 1) \times 100}{(v_{max} - 1) \times N}$$

The formula expresses the infection level in percent of strongest possible infection level

n = number of occasions at certain level

v = infection levels 1–6

v_{max} = infection level 6

N = total number of leaves or fruits studied

Number of infection levels should be kept as low as possible.

4.3. Observations on Phytotoxicity

The type and extent of such damage should be described and expressed, if appropriate, in percent terms. Detailed information on the type of damage should be given. Effects on fruit set should also be assessed.

4.4. Qualitative and/or Quantitative Recording of Yield

The weight and grade of fruits should be recorded for each plot (if possible each plant) at each picking date. National grading systems should be followed.

4.5. Interpretation of Results

The results should be analysed by appropriate statistical methods. Raw data should also be included, however, and the statistical method used should be indicated.

4.6. Detrimental Effects on Beneficial Arthropods

Information about detrimental effects on wildlife and/or beneficial arthropods, especially *Encarsia formosa* and *Phytoseiulus persimilis,* should also be noted.

NORDIC GUIDELINE NO. 2

1. *BOTRYTIS CINEREA*, CUCUMBER

1.1. Pathogen
Botrytis cinerea (Pers.) ex. Fr. on cucumber in greenhouses.

1.2. Hosts
Cucumber *(Cucumis sativus)*
(Botrytis cinerea is a polyhagous pathogen causing diseases in both ornamental and vegetable plants in greenhouses).

1.3. Symptoms
Normaly only the conidial stage of the organism is seen. The fungus causes lesions of stems, fruits and leaves. The lesions develop a grey mycelium with profuse production of hyaline conidia on branched condiophores. Stem lesions develop stem canker which may cause plant death. Black sclerotia are normally formed in dead stem tissue.

1.4. Epidemiology
The pathogen overwinters as mycelium in dead tissue and as sclerotia in the soil in greenhouses and surrounding areas.

1.5. Possibilities for Misidentification
Stem lesions can also be caused by other fungi; *Didymella bryoniae*, (Auersw.) Rehm and *Sclerotinia sclerotiorum*. (Lib.) de Bary.

2. EXPERIMENTAL CONDITIONS

2.1. Selection of Crop and Variety
A susceptible cucumber cultivar generally grown; one cultivar is used for the whole test.

2.2. Environmental Conditions
The trial should be carried out in a glasshouse, attention being paid to keeping culture conditions homogeneous. Separate glasshouses or separate glasshouse compartments must be used for each treatment if products with a high vapour pressure, fumigants, areosols or fogs are being tested.

2.3. Disposition and Size of Plots
Components: product(s) to be tested, reference product(s) and untreated control, arranged in a randomized complete block design alt. systematic block design. Plot size (net), at least 5–10 plants. Replicates, normally 4. Plots should be separated by buffer plants and buffer rows of a resistant cultivar. The minimum plot size given is appropriate for high and medium volume spray applications. If products with high vapour pressure, fumigants, aerosols or fogs are to be tested, separate glasshouses or glasshouse compartments must be used. In such cases, the trial may be carried out without replicates.

2.4. Inoculation
In order to ensure infection, the crop may be artificially inoculated (e.g. by spraying conidial suspension on the plants and keeping environment humid). The method used should be recorded.

3. APPLICATION OF TREATMENTS

3.1. Product(s) to be Tested
According to the trial description.

3.2. Reference Product(s)
Registered product(s) having widely proved satisfactory in practice. Formulation type and mode of action should be as close as possible to those of the product(s) to be tested.

3.3. Mode of Application

3.3.1. Type of Equipment Used
Application with currently used equipment. The system used should provide an even distribution of product on all plants in the plot. Give information on the type of equipment and operating conditions.

3.3.2. Time and Frequency of Application
According to the directions of use of the applicant. When no special requests are made, the first application will take place when the infection is first noticed. The product will normally be reapplied at 7-day intervals, at least 3 times. Alternative number and length of intervals should be used if requested. The number of applications and the date of each, as well as state of development of the plants, should be recorded.

3.3.3. Doses and Volumes
The product should normally be applied at the dosage recommended by the applicant.

Plants should be sprayed until total wetness (normally calculated at 250 – 350 ml solution on each plant). Equipment for low pressure spraying should be used in order to achieve maximal wetness. Data on concentration (%) and volume (litre/ha) should be given. In some cases the dosage may be given as a concentration combined with a volume commensurate with the state of the crop.

3.3.4. Data on Chemicals Used Against Other Pests, Diseases or Weeds
If other chemicals have to be applied, provision must be made to ensure that interference is minimal. These chemicals have to be applied uniformally on all plots and, normally, should be applied separately from the product(s) being tested and the reference product(s).

If biological control of insects or pathogens is being carried out, care should be taken that organisms are uniformally spread on all plots. Precise data on these applications must be given (time of treatment, stage of development of the plant and pesticide or organism used).

4. MODE OF ASSESSMENT, RECORDING AND MEASUREMENTS

4.1. Meteorological Data
Record temperature and relative humidity continuously during the entire test period.

4.2. Assessment of Effect of Fungicide(s) on Botrytis Infections of Cucurbits

4.2.1. Time and Frequency
The first assessment: immediately before treatment.

Intermediate assessment: at least three intermediate assessments should be made, timed according to disease development (generally just before the next treatment).

Final assessment: 7 – 14 days after the previous treatment. Plants treated with eradicant fungicide should have their nodes marked and infection level repeatedly controlled.

Date of each record should be noted as well as state of development of the plant.

4.2.2. Type

Assess the percentage of nodes infected active and supressed lesions on the stem on every plant of the plot. Assess the percentage infected developing internal – external fruit rots, damping fruits not included, minimum 2 plants each time (systematic rotation).

4.3. Observations on Phytotoxicity

The type and extent of such damage should be described and expressed, if appropriate, in percentages. Detailed information on the type of damage should be given. Effects on fruit set should also be assessed.

4.4. Qualitative and/or Quantitative Recording of Yield

The weight and grade of fruits should be recorded for each plot (if possible each plant) at each picking date. National grading systems should be followed.

4.5. Interpretation of Results

The results should be analysed by appropriate statistical methods. Raw data should also be included, however, and the statistical method used should be indicated.

4.6. Detrimental Effects on Beneficial Organisms

Information about the detrimental effects on (wildlife and/or) beneficial organisms should also be noted.

NORDIC GUIDELINE NO. 3

1. RED SPIDER MITE ON CUCUMBER IN GLASSHOUSE

1.1. Pathogen
Red spider mite (*Tetranychus urticae* Koch). The summer spider mite is yellowish green, blackish green with two dark spots on the top side of the abdomen. The female is about 0.6 mm long, the male about 0.5 mm with a slimmer body, pointed at the back. The winter spider mite is orange.

1.2. Hosts
The red spider mite is a polyphagous pest and can be found on practically all glasshouse cultures.

1.3. Symptoms
The symptoms vary from host to host and can readily be seen on the top side of the leaf in the form of large or small yellow-spotted parts. With heavy attacks, the ventral side of the leaf and the top shoots are covered with fine threads, and the plants may lose the leaves prematurely,

1.4. Epidemiology
Red spider mites overwinter as red, fertilized females on withering parts of plants and in cracks in glazing bars and walls. Induction of hibernation is caused by a length of day under 12 – 14 hours, but does not take place if the temperature is over 25 °C. After hibernation the females lay eggs, which after hatching, go through three stages (larval, protonymph and deutonymph) before the imaginal stage. Some of the stages are separated by a casting of skin, which takes place after a short resting stage.

Ability to procreate is very high and strongly dependent on the temperature. At 22 °C, development from egg to adult will take about 12 – 14 days. Each female lays about 120 eggs.

1.5. Possibilities for Misidentifications
None.

2. EXPERIMENTAL CONDITIONS

2.1. Selection of Crop and Variety
Cucumber (*Cucumis sativus*) should be used, and only plants of the same sort and age.

2.2. Enviromental Conditions
The trial has to be carried out in plants which are either bedded out or in pots.

Bed Plants
Good and homogeneous experimental conditions have to be obtained, especially regarding the grade of infection. Artificial infection can be achieved by transferring infected leaves to the experimental plants 8 – 14 days before the trial begins.

If the trial is carried out in nurseries, where pesticide sensitivity of the spider mites may vary, there ought to be trial in pots with spider mites with normal acaricide sensitivity.

Plants in Pots
The plants are infected when they have developed 4 – 5 character leaves. Spider mites with normal pesticide sensitivity are used. Infection should take place 8 – 14 days before the trial begins.

2.3. Disposition and Size of Plots
The trial includes an untreated experimental plot, at least one plot for every pesticide to be tested, and a plot with a standard pesticide.

Bed Plants
The trial is carried out either as block experiments, arranged in a randomized complete blocks design, each pesticide being represented by 1 plot in each block; or as a row experiment with firm plot distribution.

There should be at least 4 replications.

The size of the plot depends on local conditions and technical equipment, but it should consist of at least 10 plants. Plots should be separated by buffer plants. The minimum plot size given is suitable for high and medium volume spray applications. If products with a high vapour pressure, fumigants, aerosols or fog are being tested, separate glasshouse compartments must be used. In such cases, the trial shall be done without replicates.

Plants in Pots
Five plants per experimental plot are used and every plant constitutes 1 replication. The plants are placed as uniformly as possible without touching one another.

3. APPLICATION OF TREATMENTS

3.1. Product(s) to be Tested
According to the trial description.

3.2. Reference Product(s)
Registered product(s) having widely proved satisfactory in practice. Formulation type and mode of action should be as close as possible to those of the product(s) to be tested.

3.3. Mode of Application

3.3.1. Type of Equipment Used
Application with currently used equipment. The system used should provide an even distribution on all plants in the plot. Type of equipment, nozzle and working pressure are noted.

3.3.2. Time and Frequency of Application
This appears from the individual test plan in which the directions of use of the applicant are taken into account. Spraying is carried out only once unless otherwise stated. Every treatment is dated and the crop's growth stage is noted.

Bed Plants
Spraying is carried out when a sufficient number of spider mites appears in different stages.

Plants in Pots
Spraying is carried out when at least 30 spider mites are found per leaf.

3.3.3. Doses and Volumes
The products should be applied in the dosage recommended by the applicant.

Spraying is done until run-off starts. As the spider mites cling to the underside of the leaves, it is very important to spray upwards too.

The quantity of liquid used is noted.

3.4. Data on Chemicals used against other Pests, Diseases or Weeds

Bed Plants

If other chemicals have to be applied, provision must be made to ensure minimum interference. The chemicals have to be applied uniformly on all plots and normally separately from the product(s) being tested and the reference product(s).

If biological control of insects or pathogens is being carried out, care should be taken that organisms are uniformly spread on all plots. Precise data on these applications must be given (time of treatment, stage of development of the plant, and pesticide or organism used).

Plants in Pots

Control of other insect pests must not be carried out.

4. MODE OF ASSESSMENT, RECORDING AND MEASUREMENTS

4.1. Meteorological Data

Over the whole testing period, the daily average minimum and maximum temperatures and RH are recorded.

4.2. Assessment of Effect of Acaricide on Red Spider Mites

4.2.1. Time and Frequency

Assessment takes place immediately before spraying. In experiments in bed plants leaves selected are those where a maximum of half of the leaf shows symptoms. In experiments with plants in pots, leaves with at least 30 spider mites are selected. The leaves are marked and numbered.

The same leaves are assessed 4, 7, 14 and 28 days after spraying. On the last 2 assessments the younger leaves higher up the plant are also assessed.

Every assessment is dated and the stage of growth is noted.

4.2.2. Type

Bed Plants

Five plants per plot, 2 leaves per plant are assessed. On every leaf, counting on a 5 cm^2 leaf-area is carried out where the symptoms show.

Plants in Pots

Two leaves per plant are assessed. The whole area is assessed on every leaf.

4.2.3. Type of Methods

The red spider mites (larvae, nymphs and adults) are counted using a magnifying glass with 10 – 12 times magnification. The number of living individuals per 5 cm^2 leaf area and number per leaf are registered.

For the treated experimental plots, efficiency (W) is calculated as follows (after Henderson and Tilton).

$$W = \frac{100 \times (1 - (T_a \times C_b))}{(T_b \times C_a)}$$

The formula expresses the efficiency:

T_a = Level of attack in the treated experimental plots after spraying
T_b = Level of attack in the treated experimental plots before spraying
C_a = Level of attack in the untreated experimental plots after spraying
C_b = Level of attack in the untreated experimental plots before spraying

4.3. Observation on Phytotoxicity
The type and extent of such damage should be described and, if possible, indicated and expressed in percentages. Detailed information on the type of damage should be given. The effects on fruit should also be assessed.

4.4. Qualitative and/or Quantitative Recording of Yield
Bed plants
It may be useful to record the weight and grade of the fruit from each plot (if possible each plant) at each picking date. National grading systems should be used.

Plants in Pots
Recording of the yield is not carried out.

4.5. Interpretation of Results
The results should be analysed using appropriate statistical methods. Raw data should also be included, however, and the statistical method used should be indicated.

4.6. Detrimental Effects on Beneficial Arthropods
Information about the detrimental effects on beneficial organisms should also be noted.

NORDIC GUIDELINE NO. 4

1. PEACH-POTATO APHID ON PEPPER IN GLASSHOUSE

1.1. Pathogen
Peach-Potato aphid (*Myzus persicae* Sulz.). The adult aphid is green or light red and about 2.5 mm long. Individuals with wings have a black spot at the back. Those without wings are entirely green.

1.2. Hosts
Peach-Potato aphids are polyphagous and can be found on practically all glasshouse cultures.

1.3. Symptoms
With heavy attacks, the suction of the peach aphid may cause the leaves to curl up. Honeydew and sooty mould occur on the leaves. The peach-potato aphid often lives on the ventral side of the leaves and can easily be seen without a magnifying glass.

1.4. Epidemiology
The peach-potato aphid overwinters as eggs on peach trees or as parthenogenetic individuals on many plants in glasshouses. When spring comes, the winged aphid immigrates to the summer hosts, where new colonies are formed. Their ability to procreate is very high because of virgin birth and short generation time (about 10 – 12 days).

1.5. Possibilities for Misidentification
Cucumber aphid (*Aphis gossypii* Glov.) and potato aphid (*Aulacorthum solani* Kalt.). The cucumber aphid is a little smaller than the peach aphid (about 1.5 mm long) and yellow like a lemon with black spinal tubes.

The potato aphid is a little bigger than the peach-potato aphid (about 3 mm long). It is yellowish green with a dark green part at the pondering of each spinal tube.

2. EXPERIMENTAL CONDITIONS

2.1. Selection of Crop and Variety
Pepper (*Capsicum annum*) should be used and only plants of the same sort and age.

2.2. Environmental Conditions
The trial should be carried out in plants which are either bedded out or in pots.

Bed Plants
Good and homogeneous experimental conditions have to be obtained, especially regarding the degree of infection. Artificial infection can be achieved by transferring infected leaves to the experimental plants 8 – 14 days before the trial begins.

Regular age composition of the aphids has to be obtained. If the trial is carried out in nurseries, where insecticide sensitivity of the peach aphids may vary, there ought to be a trial in pots with peach aphids whose insecticide sensitivity is known.

Plants in Pots
The plants are infected when they are 15 – 25 cm in height. Peach-potato aphids with normal pes-

ticide sensitivity are used. Infection may take place 8 – 14 days before the trial begins, but at least 50 aphids per leaf should be present at the spraying. If resistant aphids are being used in the trial, the resistance level should be stated.

2.3. Disposition and Size of Plots
The trial includes and untreated experimental plot, at least one plot for every pesticide to be tested, and a plot with a standard pesticide.

Bed Plants
The trial is carried out either as block experiments, arranged in a randomized complete blocks design, each pesticide being represented by 1 plot in each block; or as a row experiment with firm plot distribution.

There should be at least 4 replications.

The size of the plot depends on local conditions and technical equipment, but it should consist of at least 10 plants. Plots should be separated by buffer plants. The minimum plot size given is suitable for high and medium volume spray applications. If products with a high vapour pressure, fumigants, aerosols or fog are being tested, separate glasshouse compartments must be used. In such cases, the trial shall be done without replicates.

Plants in Pots
Five plants per experimental plot are used and every plant constitutes 1 replication. The plants are placed as uniformly as possible without touching one another.

3. APPLICATION OF TREATMENTS

3.1. Product(s) to be Tested
According to the trial description.

3.2. Reference Product(s)
Registered product(s) having widely proved satisfactory in practice. Formulation type and mode of action should be as close as possible to those of the product(s) to be tested.

3.3. Mode of Application

3.3.1. Type of Equipment Used
Application with currently used equipment. The system used should provide an even distribution of product on all plants in the plot. Type of equipment, nozzle and working pressure are noted.

3.3.2. Time and Frequency of Application
This appears from the individual test plan in which the directive of use of the applicant are taken into account.

Spraying is carried out only once, unless otherwise stated. Every treatment is dated and the crop's growthstage is noted.

Bed Plants
Spraying is carried out when the attack is sufficiently spread out.

Plants in Pots
Spraying is carried out when at least 30 peach-potato aphids are found per test leaf.

3.3.3. Doses and Volumes
The products should be applied in the dosage recommended by the applicant.

Spraying is done until run-off starts. As the peach-potato aphid often clings to the underside of the leaves, and the leaves bending downwards, it is very important to spray upwards too.

The quantity of liquid used is noted.

3.4. Data on Chemicals used against other Pests, Diseases or Weeds
Bed Plants
If other chemicals have to be applied, provision must be made to ensure minimum interference. The chemicals have to be applied uniformly on all plots and normally separately from the product(s) being tested and the reference product(s).

If biological control of insects or pathogens is being carried out, care should be taken that organisms are uniformly spread on all plots. Precise data on these applications must be given (time of treatment, stage of development of the plant, and pesticide or organism used).

Plants in Pots
Control of other insect pests must not be carried out.

4. MODE OF ASSESSMENT, RECORDING AND MEASUREMENTS

4.1 Meteorological Data
Over the whole testing period, the daily average minimum and maximum temperatures and RH are recorded.

4.2. Assessment of Effect of Insecticide on Peach-Potato Aphids

4.2.1. Time and Frequency
Assessment takes place immediately before spraying. Leaves with at least 30 aphids are selected. The leaves are marked and numbered. The same leaves are assessed 1, 3 and 14 days after spraying. Every assessment is dated, and the stage of growth is noted.

4.2.2. Type
Bed Plants
Five plants per plot, 2 leaves per plant are assessed. The whole area is assessed on every leaf.

Plants in Pots
Two leaves per plant are assessed. The whole area is assessed on every leaf.

4.2.3. Type of Methods
The number of living aphids is counted on the whole leaf and registered.

For the treated experimental plots, efficiency (W) is calculated like this (after Henderson and Tilton).

$$W = \frac{100 \times (1 - (T_a \times C_b))}{(T_b \times C_a)}$$

The formula expresses the efficiency:

T_a= Level of attack in the treated experimental plots after spraying
T_b = Level of attack in the treated experimental plots before spraying
C_a = Level of attack in the untreated experimental plots after spraying
C_b = Level of attack in the untreated experimental plots before spraying.

4.3. Observation on Phytotoxicity
The type and extent of such damage should be described and, if possible, indicated and expressed in percentages. Detailed information on the type of damage should be given. The effects on fruit should also be assessed.

4.4. Qualitative and/or Quantitative Recording of Yield
Bed plants
It may be useful to record the weight and grade of the fruit from each plot (if possible each plant) at each picking date. National grading systems should be used.

Plants in Pots
Recording of the yield is not carried out.

4.5. Interpretation of Results
The results should be analysed using appropriate statistical methods. Raw data should also be included however, and the statistical method used should be indicated.

4.6. Detrimental Effects on Beneficial Arthropods
Information about the detrimental effects on beneficial organisms should also be noted.

NORDIC GUIDELINE NO. 5

1. GLASSHOUSE WHITEFLY

1.1. Pathogen

Glasshouse whitefly (*Trialeurodes vaporariorum* Westwood). The adult whitefly is about 2 mm long; it has two pairs of wings and is covered with a layer of white wax.

1.2. Hosts

The glasshouse whitefly is a polyphagous pest and can be found on practically all greenhouse cultures.

1.3. Symptoms

Small whitewinged insects fly upwards when the plants are stirred; the occurrence of honeydew (excrements) and sooty mould on the leaves.

1.4. Epidemiology

The glasshouse whitefly will exist in glasshouses throughout the year. The life cycle goes through the egg, four nymphal stages and the adult stage. Rate of development depends on temperature. At 21 °C it takes 25 – 30 days to complete one generation.

1.5. Possibilities of Misidentification

None.

2. EXPERIMENTAL CONDITIONS

2.1. Selection of Crop and Variety

Pointsettia (*Euphorbia pulcherrima*), dwarf beans (*Phaseolus vulgaris nanus*), pepper plants (*Capsicum annuum*), cucumbers (*Cucumis sativus*) or tomato plants (*Lycopersicon esculentum*) are all recommended, but in any one trial only plants of the same sort and age must be used.

2.2. Environmental Conditions

The trial should be carried out in a glasshouse with the plants grown in pots. Good homogeneous conditions are obtained at temperatures between 21 and 24 °C.

Testing of Immature Stages

For infection with eggs each plant included in the experiment is placed in a separate cage with 50 – 100 glasshouse whiteflies. After 24 hours the plants are transferred to cages free of whiteflies, care being taken to ensure that no adult whiteflies are transferred with the plant into the second cage.

Infection takes place according to time of development for the stage to be tested (see the table below):

Stage to be tested:	Egg	N1 + 2	N2 + 3	N3 + 4
Inf., day before treatment:	5	12	18	25

Testing of Adults
Single plants should be kept in separate cages during the experiment.

2.3. Disposition and Size of Plots
The trial comprises an untreated experimental plot, at least one plot for every pesticide which has to be tested, and a plot with a standard pesticide.

Five plants per experimental treatment are used and every plant constitutes 1 replication. Each of the plants is placed in an insect cage.

3. APPLICATION OF TREATMENTS

3.1. Product(s) to be Tested
According to the trial description.

3.2. Reference Product(s)
Registered product(s) having widely proved satisfactory in practice. Formulation type and mode of action should be as close as possible to those of the product(s) to be tested.

3.3. Mode of Application

3.3.1. Type of Equipment Used
Application with currently used equipment. The system used should provide an even distribution on all plants of the plot. Type of equipment, nozzle and working pressure should be noted.

3.3.2. Time and Frequency of Application
This appears on the individual test plan in which the recommandations for use of the applicant are taken into account.

Spraying is carried out only once unless otherwise stated. Every treatment is dated and the crop's growth stage is noted.

Testing of Immature Stages
Spraying until run-off is carried out on a certain day after infection depending on the stage of development being tested (see table in paragraph 2.2.).

Testing of Adults
The plants are sprayed and left to dry off. When dry, each plant is placed in a separate cage. The soil of the pot and the bottom of the cage are covered with a black plastic sheet, and 50 glasshouse whiteflies are released into each cage and left for 48 hours.

3.3.3. Doses and Volumes
The products should be applied in the dosage recommended for the applicant.

Spraying is carried out until run-off starts. As the glasshouse whitefly nymphae sit on the underside of the leaves, it is very important to spray upwards too.

The quantity of liquid used is noted.

3.4. Data on Chemicals used against other Pests, Diseases or Weeds
Control of other insect pests, diseases or weeds must not be carried out.

4. MODE OF ASSESSMENT, RECORDING AND MEASUREMENTS

4.1. Meteorological Data
For the whole testing period the average daily temperature and RH are stated indicating average minimum and average maximum.

4.2. Assessment of Effect of Insecticide on Glasshouse Whiteflies

4.2.1. Time and Frequency
Testing of Immature Stages
Assessment takes place immediately before spraying. Leaves selected are those on which there are at least 30 glasshouse whitefly eggs or nymphae. The leaves are marked and numbered.

The same leaves are assessed after spraying. For the control of eggs and nymphal stages one and two, this assessment is done 7 – 10 days after spraying. For the control of nymphal stages three and four, assessment is done 20 days after spraying.

Testing of Imagines
Forty-eight hours after the insect release into the cage the number of dead glasshouse whiteflies is counted. Possibly also the number of eggs laid on the plant during the 48 hours exposure.

4.2.2. Type
With eggs and nymphae at least 2 leaves per plant are assessed. The whole area is assessed on every leaf. All dead imagines are counted in each cage.

4.2.3. Type of Methods
Before spraying the number of eggs and living nymphae (stages 1 – 4) are counted using a magnifying glass of 10 – 12 times magnification. After spraying both living and dead individuals are counted per leaf under a microscope.

For the treated experimental plot the efficiency (W) is calculated like this:

$$W = \frac{B \times 100}{(A + B)}$$

A = Number of living individuals
B = Number of dead individuals
W = Efficiency

4.3. Observation of Phytotoxicity
The type and extent of such damage should be described and if possible indicated and expressed in percentages. Detailed information on the type of damage should be given. The effects on fruit should also be assessed.

4.4. Qualitative and/or Quantitative Recording of Yield
Recording of the yield is not carried out.

4.5. Interpretation of Results
The results should be analysed by appropriate statistical methods. Raw data should also be included and the statistical method used should be indicated.

NORDIC GUIDELINE NO. 6

1. FUSARIUM WILT OF CARNATION

1.1. Pathogen
Fusarium oxysporum Schl. f. sp. *dianthi* (Prill. & Del.) Snyder & Hansen.

1.2. Hosts
Carnation (*Dianthus* ssp.)

1.3. Symptoms
Shoots turn yellowish and wither. First, only a part of the plant may wilt, but finally the whole plant dies. Dead shoots are pale brown, even white at the top. Vascular tissue mid-brown at the stem base.

1.4. Epidemiology
The pathogen can survive infective in soil and subsoil for several years. It spreads through soil and root contacts from diseased plants and infested cuttings. The fungus enters the host via roots and cutting injuries. It spreads also by conidia.

1.5. Possibilities for Misidentification(s)
Other fungi and bacteria causing wilt symptoms, like *Phialophora cinerescens, Fusarium culmorum, Erwinia chrysanthemi, Pseudomonas caryophylli.*

2. EXPERIMENTAL CONDITIONS

2.1. Selection of Crop and Variety
A variety susceptible to *Fusarium* wilt, for example White Sim, is used. The same variety is used in the whole trial.

2.2. Environmental Conditions
The trial has to be made in a controlled environment. Attention should be paid to homogeneous culture conditions suitable to carnation. Trials in beds should be irrigated and fertilized automatically. Plots are isolated from the subsoil and other plots with a plastic cover. In controlled conditions the growth substrate must be free from soil-borne pathogens.

A separate glasshouse or separate compartments should be used for each treatment if products with high vapour pressure, fumigants or fogs are to be tested. When trials are made in the grower's greenhouse normal growth substrate is used.

2.3. Disposition and Size of Plots
Components: product(s) to be tested, reference product(s) and untreated control arranged in a complete block design or systematic block design. Plot size (net): 0.5 – 2.0 cm^2, approximately 40 plants/m^2. Replicates normally 4. Plots should be separated by buffer plants and buffer rows. The minimum plot size given is appropriate for high and medium volume spray applications. If products with a high vapour pressure, fumigants or fogs are to be tested, separate compartments must be used. In that case the trial should be done without replicates.

When the trial is made in the grower's greenhouse, plot size should be 8 – 20 m^2.

2.4. Inoculation
Artificial inoculation in controlled conditions can be carried out in two ways. Infested substrate, used old peat or mull, is spread 1 l/m^2 under the new substrate. Alternatively the roots of every tenth plant are dipped into suspension containing *Fusarium oxysporum* spores and mycelium before planting. The infestation method used should be recorded. When artificial infestation is used, there should also be plots which are not inoculated. When the trial is made in the grower's glasshouse artificial infestation is not necessary.

3. APPLICATION OF TREATMENTS

3.1. Product(s) to be Tested
According to trial description given by manufacturer or according to reference products.

3.2. Reference Product(s)
Registered products having widely proved satisfactory in practice. Formulation type and mode of action should be as close as possible to those product(s) to be tested.

3.3. Mode of Application

3.3.1. Type of Equipment Used
Application with currently used equipment. The system used should provide an even distribution of the product on all plants of the plot. Give information on the type of equipment and operation conditions used.

3.3.2. Time and Frequency of Application
According to the instructions of the applicant. When no special requests are made, the first application should be 0 – 2 weeks after planting. The products are re-applied at 2 – 3 month intervals so that applications take place in February, April – May, July and September – October. Alternatively the applications can be made at one month intervals beginning from planting and continuing through out the whole growing season. The number of applications and the date of each application as well as state of development of the plants, should be recorded.

3.3.3. Doses and Volumes
The product(s) should normally be applied at the dosage recommended by the applicant. The application is made with 2 – 3 l of solution/m^2 if no other requests are made. Data on concentration and volume should be given.

3.3.4. Data on Chemicals used against other Pests, Diseases and Weeds
If other chemicals have to be applied, provision must be made so that they cause minimum interference. These chemicals have to be applied uniformly on all plots and separately from the products to be tested and the reference products. If biological control of insects or pathogens is used, care should be taken that organisms are uniformly spread on all the plots. If the product to be tested is a biological control agent, its susceptibility to pesticides should be known. Precise data on all applications must be given (time of treatment, stage of development of the plant and pesticide or organism used).

4. MODE OF ASSESSMENT, RECORDING AND MEASUREMENTS

4.1. Meteorological Data
Record temperature and relative humidity continuously during the entire test period.

4.2. Assessment of Effect of Fungicide(s) on Wilt Disease of Carnation

4.2.1. Time and Frequency
Dying and severely diseased plants are removed from the plots and counted during the whole trial. At the end of the trial the plants are graded according to their disease level. The plot area covered by diseased plants is approximated before treatments during the whole trial and at the end of the trial. The time and data of the measurements are recorded.

4.2.2. Type
In the assessment the plants are graded from 1 to 5 according to disease symptoms.

5: dead or nearly dead
4: visible symptoms in shoots
3: vascular tissue brown at the basal parts of the plant, no visible wilt symptoms
2: vascular tissue green, but *Fusarium oxysporum* growing in agar culture
1: healthy plant, no fungal growth on agar plate

Isolation of the pathogen from stem bases is made on cornmeal agar supplemented with 200 ppm streptomycin sulphate. Isolation is made of the plants showing no symptoms.

From trials made in the grower's greenhouse the amount of disease is estimated as the proportion of dead plants from the whole plot area.

For the treated experimental plots the efficiency (P) after Townsend and Heuberger is calculated like this:

$$P = \frac{(n \times (v - 1)) \times 100}{(^{v}max\ 1) \times N}$$

The formula expresses the infection level in percent of strongest possible infection level.

n = number of occasisons at certain level
v = infection levels 1 – 5
^{v}max = infection level 5
N = total number of plants studied

4.3. Observations on Phytotoxicity
The type and extent of such damage should be described and expressed if appropriate, in % terms. Detailed information on the type of damage should be given. Effects on flowering should also be assessed.

4.4. Qualitative and/or Quantitative Recording of Yield
The number of flowers is counted in every plot. The flowers are graded by quality according to national grading systems. The beginning of flowering is also recorded.

4.5. Interpretation of Results
The results should be analysed by appropriate statistical methods. Raw data should, however, also be included and the statistical method used should be indicated.

4.6. Detrimental Effects on Beneficial Organisms
Information about detrimental effects on wildlife and/or beneficial organisms should also be noted.