Reference Manual A-Section 3.2

3.2 PHYTOSANITARY FIELD INSPECTION PROCEDURES

REVISION HISTORY: Version 1.2: Disease Diagnosis in the Field #4.. Disease Diagnosis in the Lab #2 , Reports page 5
Version 1.3 Document formatting update-04 04 2019, change in numbering no content changes

3.2.1 Equipment Requirements

1. Field maps
2. 10x hand lens
3. Pocket knife/scissors
4. Tape measure
5. Sampling bags or envelopes (paper)
6. Labels
7. Ice chest
8. Hand counter
9. Diagnostic illustrations
10. Report forms

3.2.2 Field Inspection Strategy

3.2.2.1 Field Overview

Find a point near the field that allows the best opportunity to look the field over. This overview enables:

a. Verification that the field is correctly identified by comparing the map or information provided and the observed field.
b. Identification of special areas or microclimates in the field that appear different enough to warrant special attention when inspected. These would include:

3.2.2.1.1 *Locations in which high moisture levels may be retained such as proximity to:*

Rivers and streams
Drainage areas
Low spots
Weedy areas

3.2.2.1.2 *Areas of the field affected by borders, such as:*

Field edges
Tree lines in the field
Adjacent fields of a similar crop

Presence of buildings or bins

3.2.2.1.3 *Drought stress areas, such as:*
High spots
Light textured soils
Margins or overhead irrigation area

3.2.3 **Establish the Seed Field Inspection Pattern**

3.2.3.1 The seed field inspection pattern should ensure that all parts of the field are adequately and proportionately represented in the plants inspected within the various micro- climates of the field.

3.2.3.2 As long as these requirements are met, the pattern of field inspection can vary.

3.2.3.3 Examples of established inspection patterns are as follows. Other formats may, however, be acceptable.

3.2.3.3.1 **Stagger "X" pattern.** (CDFA Phytosanitary Certification Manual, 1985)
This is used for cereal crops and requires examination of plants along one side of the field, then diagonally in a stagger pattern across rows to the far corner, across the far side, and diagonally back to starting corner (Figure 1). Additional examinations may be necessary for field environments not covered by the inspection pattern.

3.2.3.3.2 **Equidistant passes pattern.** (CDFA Phytosanitary Certification Manual, 1985)
This system is used for crops other than cereals. Table 1 lists the minimum number of field passes (Figure 2) in relation to field size to give a minimum of 95% confidence level in detecting an infection level of 0.1%.

3.2.3.3.3 **Customized field inspection pattern.**
This system allocates appropriate numbers of plants to be inspected in the various environments in a field. An example is shown in Fig 3.

Table 1.
Minimum field passes per acre.

Field size (acres)	Minimum # passes
0 - 1	6
1 - 5	9
5 - 10	11
10 - 20	13
20 - 50	17
50 - 100	20
100 - 200	24
200 - 500	30
500 – 1000	36

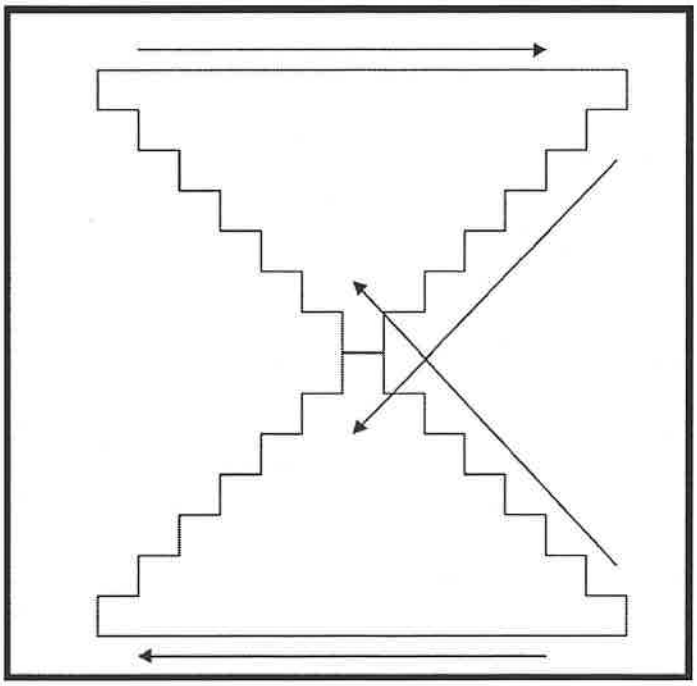


Figure 1. "X" Field Inspection Pattern

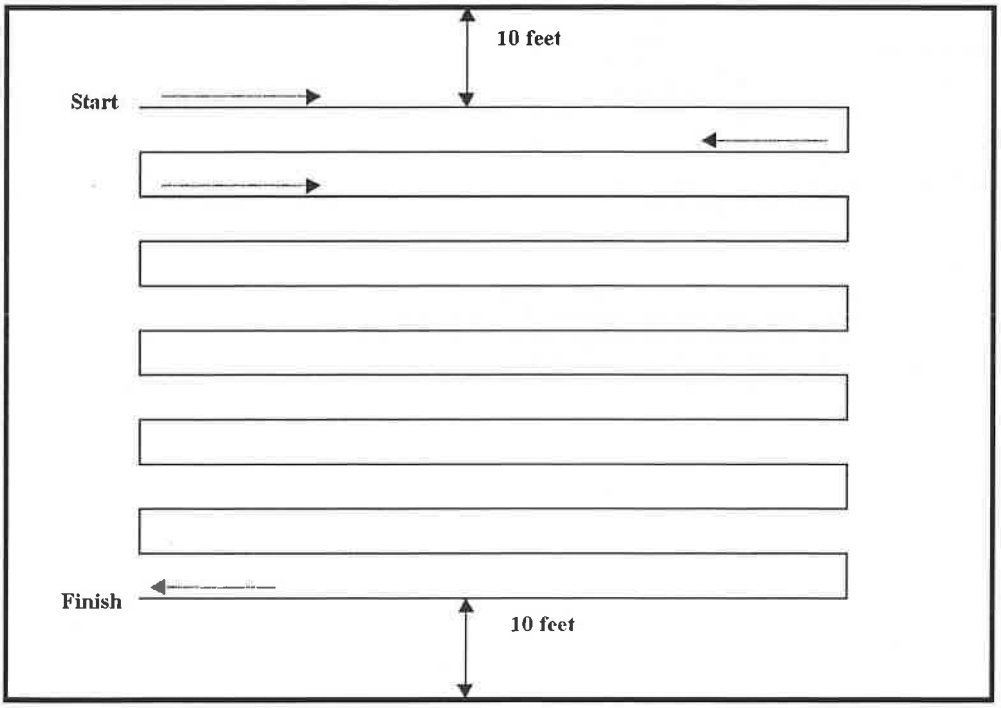
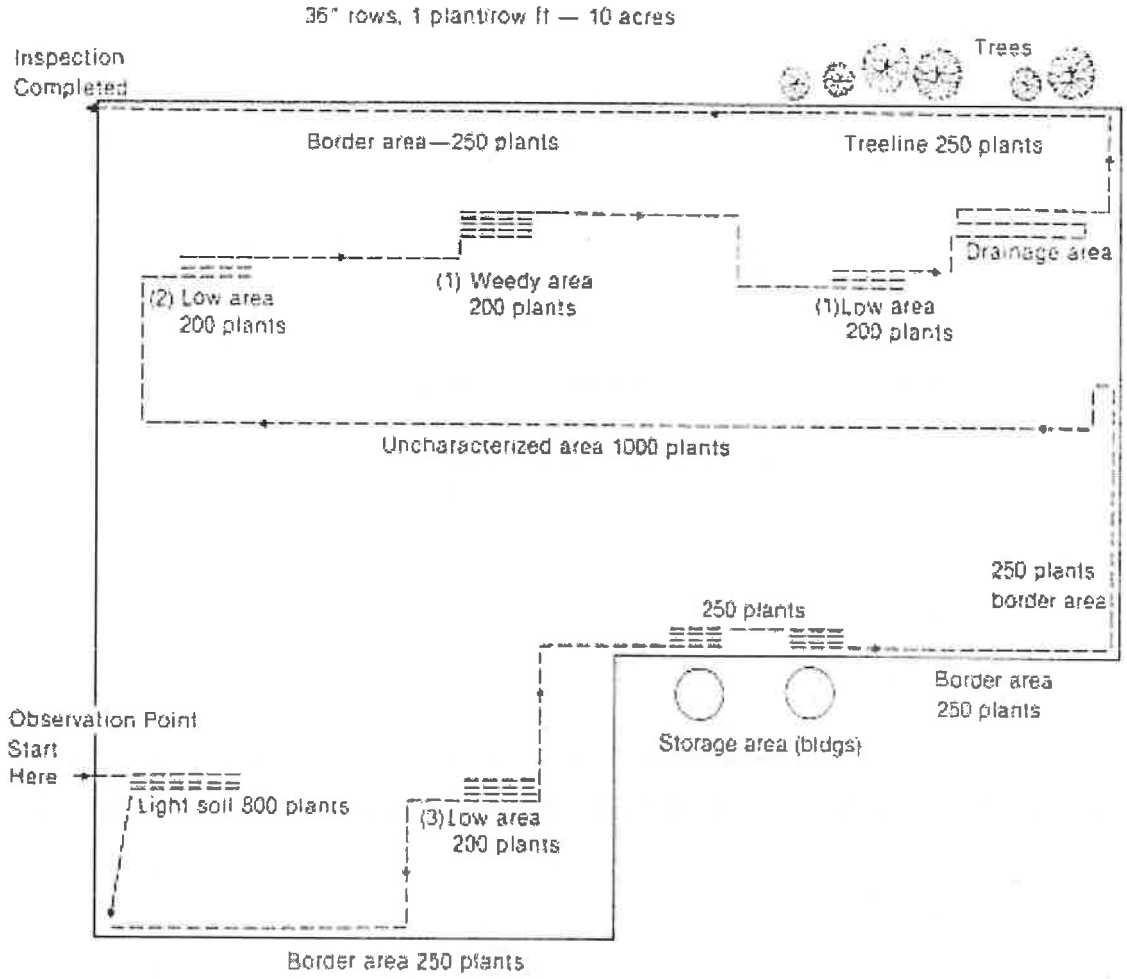


Figure 2 - Equidistant Pass Pattern

Figure 3. Example of field inspection by customized pattern



This is an example of how a field inspection map might look. Notice that the inspector has shown the field shape, areas inspected, number of plants inspected in each area, code for each special area (example, low areas 1, 2, 3) and inspection pattern.

3.2.4 **Disease Diagnosis in the Field**

3.2.4.1 The presence or absence of diseases relevant to the inspection requirements is first determined by visual examination of plants in the field. Descriptions of signs and symptoms are provided in this manual for the individual diseases of the major seed crops. Other established aids to identification may also be used.

3.2.4.2 Inspections have to be made at crop growth stage when signs or symptoms of a disease are likely to be present. Appropriate inspection times for particular pests or diseases are indicated in this manual.

3.2.4.3 An appropriate number of plant samples, representative of diseases in the field, should be taken for laboratory confirmation of the visual diagnosis. More extensive samplingshould be carried out when visual symptoms are insufficient to ensure to accurate diagnosis. Samples of suspected disease tissue should be kept flat in paper envelopes or towels in a plastic bag in ice chest. All samples should be correctly labeled to indicate date, time, locations, crop, and plant part.

3.2.4.4 Diseases caused by regulated pathogens must be sampled, as described above, and confirmed by appropriate laboratory analysis under the supervision of a trained plant pathologist. Accredited entities may use internal diagnostic capabilities or a qualified 3rd- party lab, such as National Plant Diagnostic Network Laboratory.

3.2.5 **Disease Diagnosis in the Laboratory**

3.2.5.1 Accredited Entities using a 3rd-party lab for field sample diagnosis must have an agreement in writing acknowledging that the lab agrees to diagnose samples for the National Seed Health System.

3.2.5.2 Samples should be processed systematically in a laboratory facility with demonstrated proficiency in diagnosing plant diseases. Accredited entities using internal diagnostic labs must have appropriate facilities, plant pathology expertise, and training procedures, which will be evaluated during accreditation audits.

3.2.6 **Reports**

Inspection reports should be made on a standard form similar to the example provided below. Accredited entities are encouraged to seek input from state plant regulatory officials in the development of appropriate report forms.

PHYTOSANITARY GROWING SEASON INSPECTION REPORT

Crop _____ _____ Accredited Entity

PLEASE PRINT

_____ Company Name _____ Variety

_____ Company Contact Official _____ Field # Acres

_____ Address TYPE OF FIELD: Increase/Production

_____ Telephone Number

_____ Contract Grower Phone _____ County

Growth Stage & Date: _____ 1st Insp. _____ 2nd Insp. _____ 3rd Insp.

INSPECTION DATA

(Refer to list of plant diseases/pests on separate p a g e s)

Code	Severity (Optional) Low, Moderate, High L, M, H	Lab Sample Submitted Yes	No	Lab Confirmation Field Diagnosis Confirmed Yes/No	Additional Pathogens Identified Code	Lab Sample Number
___	_____	[ ]	[ ]	[ ]	_____	_____
___	_____	[ ]	[ ]	[ ]	_____	_____
___	_____	[ ]	[ ]	[ ]	_____	_____
___	_____	[ ]	[ ]	[ ]	_____	_____
___	_____	[ ]	[ ]	[ ]	_____	_____
___	_____	[ ]	[ ]	[ ]	_____	_____
___	_____	[ ]	[ ]	[ ]	_____	_____
___	_____	[ ]	[ ]	[ ]	_____	_____

☐ No Other Diseases Noted.

I inspected these fields during active growth and determined the above diseases/pests were found as indicated.

Remarks

INSPECTOR_____ID NUMBER:_____DATE:_____

LAB MANAGER_(If sample submitted)_____ID NUMBER:_____DATE:_____

Diagnostic Aids for Phytosanitary Field Inspections

Pantoea stewartii (syn. *Erwinia stewartii*)

Time of inspection:
Corn plants in early flowering stage

Field symptoms:
Leaf: Leaves show linear, pale-green to yellow streaks with irregular or wavy margins that run parallel to veins and may extend the length of the leaf (Figure 1). These streaks soon become dry and brown. Masses of bacteria may also stream from the cut edges of infected leaf tissue. When diseased tissue was placed near the edge of a water drop, the drop quickly became cloudy.

Stalk: Conducting vessels become plugged with bright-yellow slime. If infected stems are cut in cross section, the yellow slime will often exude. Cavities form in the stalk near the soil line in severely infected plants.

Whole plant: Plants infected early in the season may show wilting and stunting (Figure 2).

Indicators of disease presence:
The corn flea beetle, *Chaetocnema pulicaria*, is generally recognized as the most important carrier of inoculum for *E. stewartii* in the USA (Pepper, 1967). The pathogen overwinters in the alimentary tract of this insect, which emerges from hibernation and feeds on young maize. The occurrence of substantial numbers of the corn flea beetle at the beginning of the growing season (Figure 3) and subsequent feeding scars of this insect on leaves of the growing corn plant are strong indictors of infection by *E. stewartii*.

In the US Corn Belt, survival of the corn flea beetle is greatly reduced by low winter temperatures. Severity of Stewart's wilt can be forecast on the basis of the average temperatures during December, January, and February.

Laboratory diagnosis:
Leaf sections with suspect Stewart's wilt lesions may be cut across veins and observed under the microscope for bacterial streaming from the vascular tissue.

Suspect colonies of *E. stewartii* on culture medium can be tested for pathogenicity on susceptible seedlings.

The presence of the *E. stewartii* in plant tissue can also be detected with an ELISA test kit (Agdia. Elkhart, Indiana). This kit is adapted from the method of Lamka et al, 1991). The method, described in Method 2.1 in Section 1 of this manual for detection of seedborne *E. stewartii,* can also be used to detect the pathogen in leaf or stalk tissue and in insects.

References:
Lamka GL, Hill JH, McGee DC, and Braun, EJ. 1991. Development of an immunosorbent assay for seed-borne *Erwinia stewartii*. Phytopathology 81:839-846.

Pepper EH, 1967. Stewart's Bacterial Wilt of Corn. Monogr. 4. St. Paul, Minnesota, USA: American Phytopathological Society.

Alternaria dauci

Time of inspection:
Buds on carrot plants are just beginning to flower and tops still green

Field symptoms:
Leaf: Lesions produced on leaf and petiole tissues are generally dark-brown to black, and chlorosis of surrounding tissues is observed. Gradually, the spots increase in size and become confluent. Finally, the whole leaf becomes grayish-black, while the leaflets become curly and convolute. The older leaves are more heavily infected than the young ones (Figure 2).

Stalk: The stem bark is discolored.

Root: Root lesions are irregular in shape, dark-brown to black. The decay is dark-brown to black, firm and shallow.

Floral structures: *Alternaria dauci* causes dark, longitudinal spots on flower-stalks and umbels, and attacks flowers and immature seeds, causing them to be discolored (Figures 3 & 4).

Whole plant:
When infection is severe, the top part of the may be killed (Figure 1).

Indicators of disease presence:
The pathogen requires the presence of moisture for infection. Heavy dews and rains are favorable for this process. The optimum temperature for infection is 82°F.

Laboratory diagnosis:
For lesions on plant parts, incubation in a moist chamber on blotters at 20-25°C or on agar media will lead to the production of conidiophores and conidia for final identification (Figures 5 & 6). The methods of isolation of *A. dauci* from diseased carrot leaves, seedlings and seeds and the ways to achieve abundant sporulation are described in detail by Strandberg (1987).

References:

Strandberg JO. 1987. Isolation, storage, and, inoculum production methods for *Alternaria dauci*. Phytopathology. 77(7):1008-1012.