
Foreign Leaves in Oregano

Purpose: To determine the presence of foreign leaves in oregano.

A. Apparatus

1. Stereoscopic binocular microscope--widefield with the following minimum specifications: 3 parfocal objectives--1X, 3X and 6X or 7.5X; paired 10X widefield oculars, mounted on a base and capable of illumination by transmitted or reflected light. Ordinarily 30X magnification is used for routine examination of filter papers. Confirmation of suspect material at higher magnification may be required.
2. Compound polarizing microscope - microscope with the following minimum specifications: 4 parfocal achromatic objectives of ca 4, 10, 20, and 40X; revolving 4-place nosepiece; Abbe condenser with N.A. of 1.25; 10X Huygenian or widefield eyepieces; fine adjustment; mechanical stage; fitted with polarizing prisms below and above the mechanical stage.
3. 100 mm Petri plates.
4. 7 cm (or 9 cm) ruled filter papers.
5. Microscope slides and cover slips.
6. Eye dropper.
7. Dissecting needle and forceps.

B. Reagents

1. Xylene - reagent grade.

C. Preparation of Sample:

1. Utilize sample procedures for original sampling (see Method 14.1). From each sub-sample, take a representative 10 g (approximately 75 mL) sample of oregano leaf. These samples will be composited and gently blended.
2. Prepare a 5 to 10 g analytical sample from above using a sampling splitter or a quartering procedure.
3. Maintain leaf integrity; do not break up leaves.

D. Procedure

1. Gently shake enough oregano into one Petri plate lined with standard 7 cm (or 9 cm) ruled filter paper to cover the filter paper in a single layer (approximately 500 leaf fragments per plate; in no case is less than 400 mg of oregano fragments to be viewed).

2. Examine the plate of material under the 30X to 60X magnification of the stereoscopic binocular microscope looking for any leaf fragment other than oregano. (See notes 1 and 2).
3. If no sumac is found in the plate it can be reported as “Sumac Negative”.
4. If foreign leaf fragments are observed place oregano leaf fragments and representatives of any foreign leaf fragments on a glass microscope slide and place a cover slip over the material. Using an eye dropper, introduce enough xylene to fill the cover slip.
5. Examine the slide using the compound polarizing microscope using both crossed and uncrossed polars.
6. If sumac is detected and confirmed, report as “Sumac Positive”.
7. If any other foreign leaf is detected estimate the total percentage and add to extraneous matter total.

E. Calculation

N/A

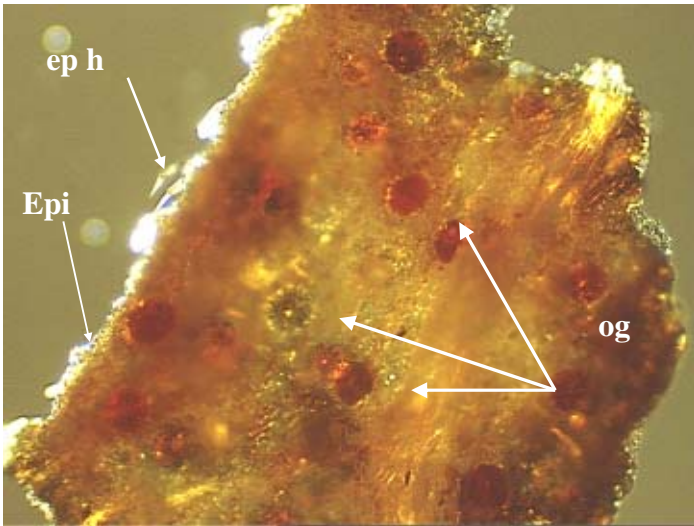
F. Statistics

N/A

G. Notes

Upon removing the polarization, the oxalate crystals in the sumac disappear.

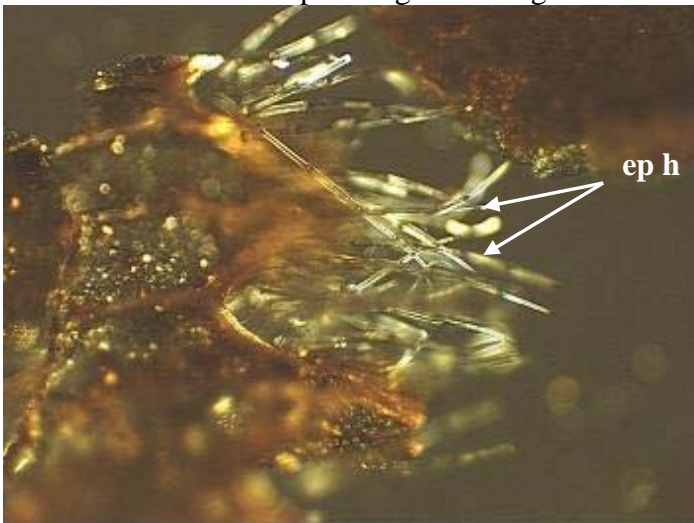
1. Oregano leaves contain numerous large red to red/orange oil glands evenly distributed over leaf surface. The leaf surface is rough, waxy and scale-like. Numerous jointed, curved, pointed, multicellular hairs are present. No oxalate crystals are present.



Microscopic *Oreganum vulgare* L



Oreganum vulgare



Microscopic *Origanum onites*



Origanum onites

2. The following foreign leaves have been found in oregano:

- **Sumac:** The leaf surfaces are smooth and hairy with no heavy waxy coating. Epidermal hairs are long, sharply pointed, slightly curved and have a conspicuous central hollow canal. The hairs show birefringence when viewed under crossed polars. No oil glands are visible on the leaf surface. When viewed under crossed polars, the leaf shows numerous clusters of calcium oxalate crystals which are interspersed throughout the leaf and are heavily clustered along leaf veins.

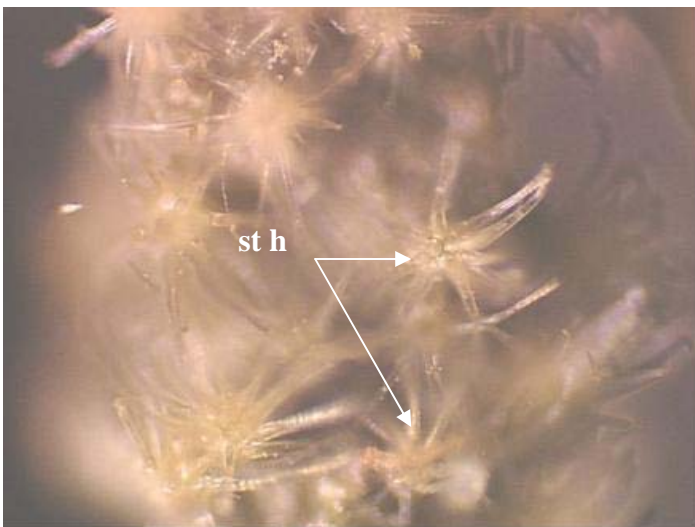


Microscopic Sumac



Sumac

- **Cistus spp.:** The undersides of the leaves contain many multi-branched epidermal hairs. No oxalate crystals present.

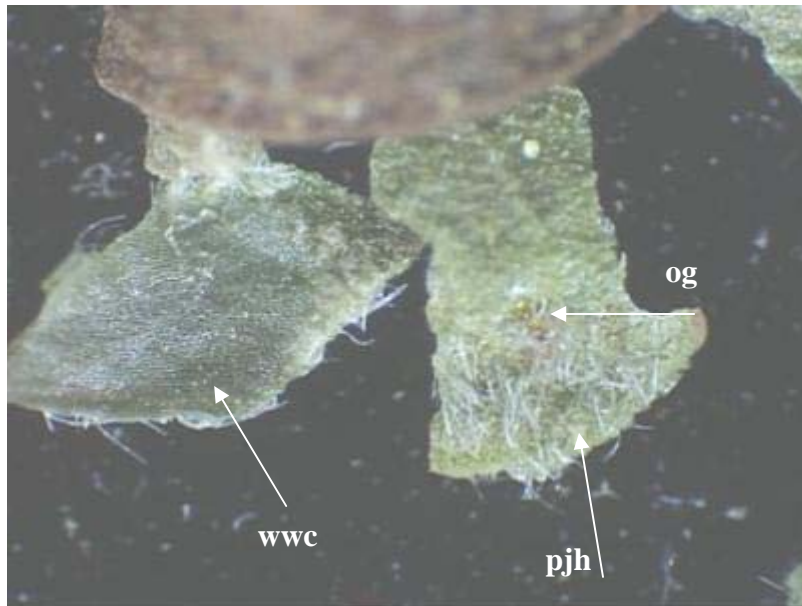


Microscopic Cistus

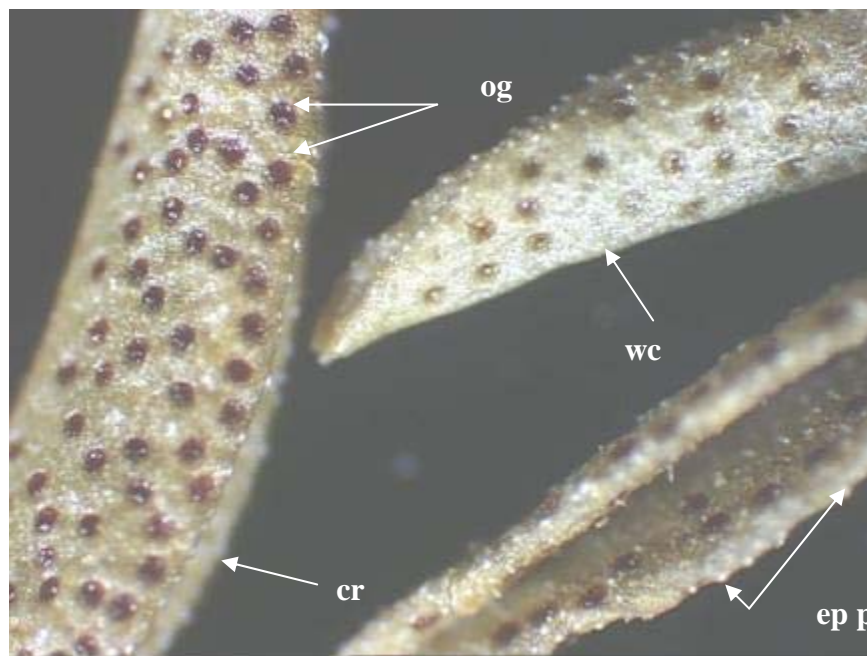


Cistus

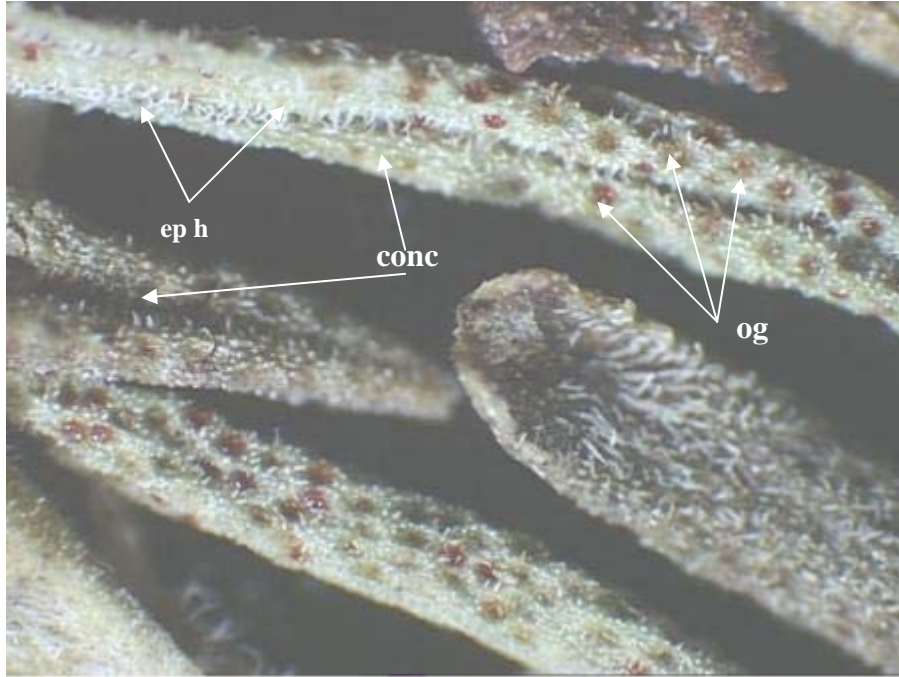
- **Marjoram:** Oil glands are widely scattered and are a bright yellow-gold colored. The glands are usually smaller and more irregularly placed across the leaf surfaces than oil glands on the oregano leaf. Surface hairs are long, pointed, multi-celled and often in dense clusters.



- **Savory:** Can be distinguished by the extreme inward to upward folding of the leaf along the midrib. The cuticle is characterized by a tough, waxy material and short, stubby, capitate epidermal projections. The oil glands are almost as large as those on oregano leaves except that there are many more of them. They are very evenly placed on the leaf, are orange to slightly brown in color, and may even be collapsed.



- **Thyme:** the leaves are oval to lance-shaped or needle-like in overall appearance. The leaf surface is waxy and many small greenish to orange-red oil glands are visible. These are more numerous but not nearly as large or as red as those seen on oregano leaves. There is a concavity or fold which traverses centrally the entire length of each leaf. Surface hairs are numerous, short curved and not as obviously jointed as those on oregano leaves.



KEY

ep h – epidermal hairs	pjh – pointed, jointed hairs
epi – epidermis	wwc – wavy-walled cells
og – oil glands	cr – crease (along midrib)
st h – stellate epidermal hairs	wc – waxy coating
conc - concavity	cu - cuticle

- **Hemlock:** The epidermis bears stomata on both its surfaces, but much more abundant on the under than on the upper. The cells of the upper epidermis have wavy walls, while those on the under epidermis, which are smaller, are sinuate and slightly striated. There are no oxalate crystals.

- **Olive:** The epidermis has a rough wax layer and abundant stomata. A few stellar trichomes cover the upper epidermis. The bottom leaf surface has a silvery color. The leaf appears thicker than others. There is a dimple-like appearance of the upper epidermis.



- **Other:** hazelnut, myrtle, strawberry tree



Hazelnut



myrtle



wild strawberry tree leaves

H. References

ASTA Manual: Microscopic Identification of Spices (2003)

I. Revision History

10/03/10 Added instructions for detection of the following foreign leaves: cistus, marjoram, savory, thyme, hemlock, olive. Added reference to ASTA manual on Microscopic Identification of Spices.