Bud Knot: A Disorder of Bergamot*

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ABSTRACT. A virus-like disorder of bergamot, known as bud knot, is described. The symptoms are bud enlargement, shortened internodes, yellowing and corking of the leaf veins, dieback of stems and branches. Transmission and propagation tests, histology, and *in vitro* culture suggest that it is a propagable, non-infectious disorder. The perpetuation of some symptoms through seeds is a further suggestion of a genetic origin.

Index words. Genetic disorder, tumour-growth, histopathology.

The origin of bergamot is not known. Some attribute it to China or the West Indies, whereas others do not exclude the possibility that it began as a citrus mutation in the Calabria region of southern Italy, and others believe that it is a cross between sour orange and lemon.

Bergamot groves have been known in Calabria for more than three centuries for the essences obtained from the peel, the flowers and the leaves, which are used for the best perfumes. At present, about 2,500 hectares are planted.

Many diseases affect bergamot trees causing variable damage (3, 4, 9), which have not been well evaluated. Among these is a virus-like disorder first noticed on a few trees and described as bud knot (5). Since in later surveys, the disorder appeared widespread a study was undertaken to investigate its etiology.

SYMPTOMS

The most typical symptom of the disease is a bud enlargement which appears similar to that induced by the mite *Eriophyes sheldoni* Ewing on lemon buds. Twigs 1 to 3 years old show a remarkable increase in size of apical and lateral buds which may double the normal diameter (fig. 1). When apical buds are affected the internodes are shortened and stem growth stops. In older stems the knots are less evident or disappear. Leaves coming from enlarged buds have vein yellowing and a pale color. Sometimes a vein corking very similar to that induced by boron deficiency is also observed (fig. 2). A few twigs, stems, branches or the entire canopy may be affected. Leaves drop prematurely and a stem dieback may occur suddenly. Seriously affected trees show yellowing of affected branches and poor growth, but they recover after removal of abnormal stems and proper cultivation. In some cases pruning of limbs induces abundant growth of cicatricial tissue which repair the wound and appear like a tumor.

Castagnaro and Fantastico varieties, which are more vigorous, show more severe symptoms, but the Femminello variety is also affected.

The disorder is widespread throughout the growing area and probably affects every tree, regardless of variety.

TRANSMISSION AND PROPAGATION TESTS

Bark patches taken from the internodes of affected stems and twigs were grafted on seedlings of sour orange, Volkamer lemon (fig. 3) and Mexican lime growing in a greenhouse at 21-35C. No symptom of vein enation or tristeza

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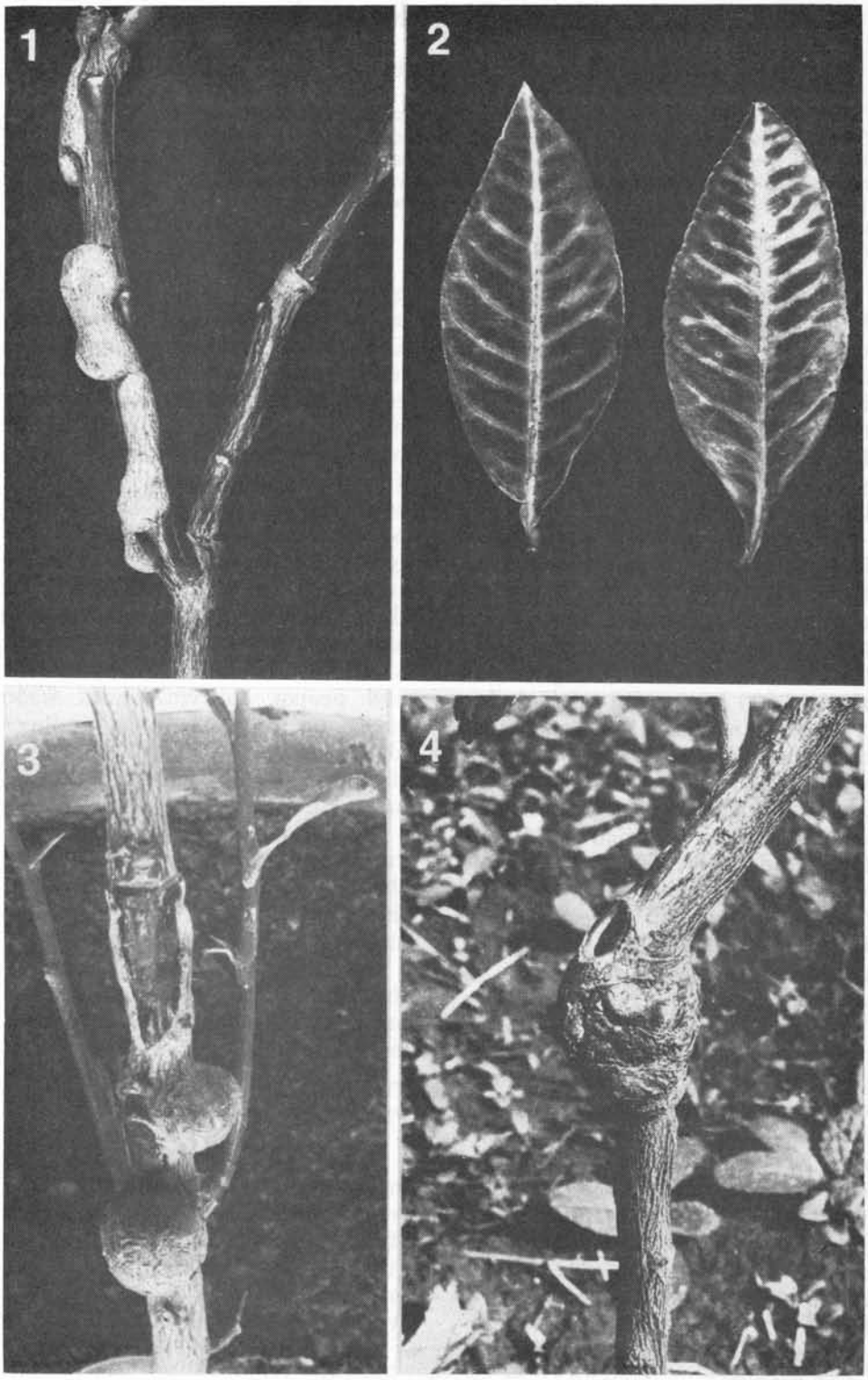
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was observed on the young flushes observed for one year; psorosis young leaf symptoms appeared on some tested plants. In many cases, a spheroidal tumor appeared along the union line between the inoculum patch and the test seedling (fig. 4). They enlarged up to 0.8 cm in diameter. Wounds of the bark above and below the inoculation point, made with a pin point or a knife, did not stimulate an abnormal reaction.

Knotted buds grafted on sour orange seedlings produced an abnormal overgrowth. One of these plants was transplanted to the field and, after 15 years grew poorly. It is about 1.20 m tall and an extraordinary overgrowth formed at the budunion (fig. 5). The circumference of the tumor is 29 cm, whereas 10 cm above, the scion is 8 cm and the stock is 9 cm. Leaf symptoms are very mild.

Topworking buds from that tree to an old Avana mandarin tree on sour orange gave the same results as above. After only 1 year the budunion had an overgrowth and some spheroidal tumors developed from the upper buds (fig. 6). Later, the branches showed leaf yellowing, vein corking and stem dieback. No symptom appeared on mandarin sprouts.

Young bergamot trees (2 years old) obtained by grafting buds from a commercial orchard on sour orange stock displayed a variable difference in diameter between scion and stock. On the contrary, little or no difference in growth was observed when buds from 2-year-old stems were propagated. Trees of different ages, up to 40 years, in commercial groves have only a small overgrowth of the scion. Seeds of the Femminello variety produced seedlings with some morphological differences because of the monoembriony of this species. After 1 year none of them shows clear cut symptoms, but some have vein corking and uneven growth of the apical portion.

HISTOLOGICAL OBSERVATIONS

Young stems (about 1 cm in diameter), with and without bud knots were cut in pieces of few millimeters each, fixed in ethyl alcohol-acetic acid (3:1) and embedded in paraffin. Sections 20 μ thick were cut with a microtome and stained with a 0.5% quinacrine solution. After rinsing in alcohol, sections were mounted in water and examined under a Zeiss microscope equipped with a fluorescent source.

Stem with knots showed altered sectors with hypertrophic xylem. Few or no vessels were present in the outer layers. Woody elements were disorganized (fig. 7). No modification was observed in the phloem. On the side opposite the bud and in the internode, the xylem appeared normal, without any modification of its elements (fig. 8).

IN VITRO TISSUE CULTURE

Green nodes and internodes of non-suberized twigs were taken from affected plants and used as explant source for the *in vitro* tissue culture. Etrog citron twigs were used as controls (8) because of the lack of a healthy bergamot tree. The leaves and thorns were cut off and the sticks were washed

Fig. 1. Twigs of bergamot showing apical bud knots and shortened internodes.

Fig. 2. Yellowing and corking of midveins of leaves from bergamot with bud knot.

Fig. 3. Spheroidal tumors along the budunion line on Mexican lime seedlings eight months after grafting of bark patches taken from internodes of bergamot affected by bud knot.

Fig. 4. Overgrowth of scion in a 2-year-old bergamot tree on sour orange rootstock.

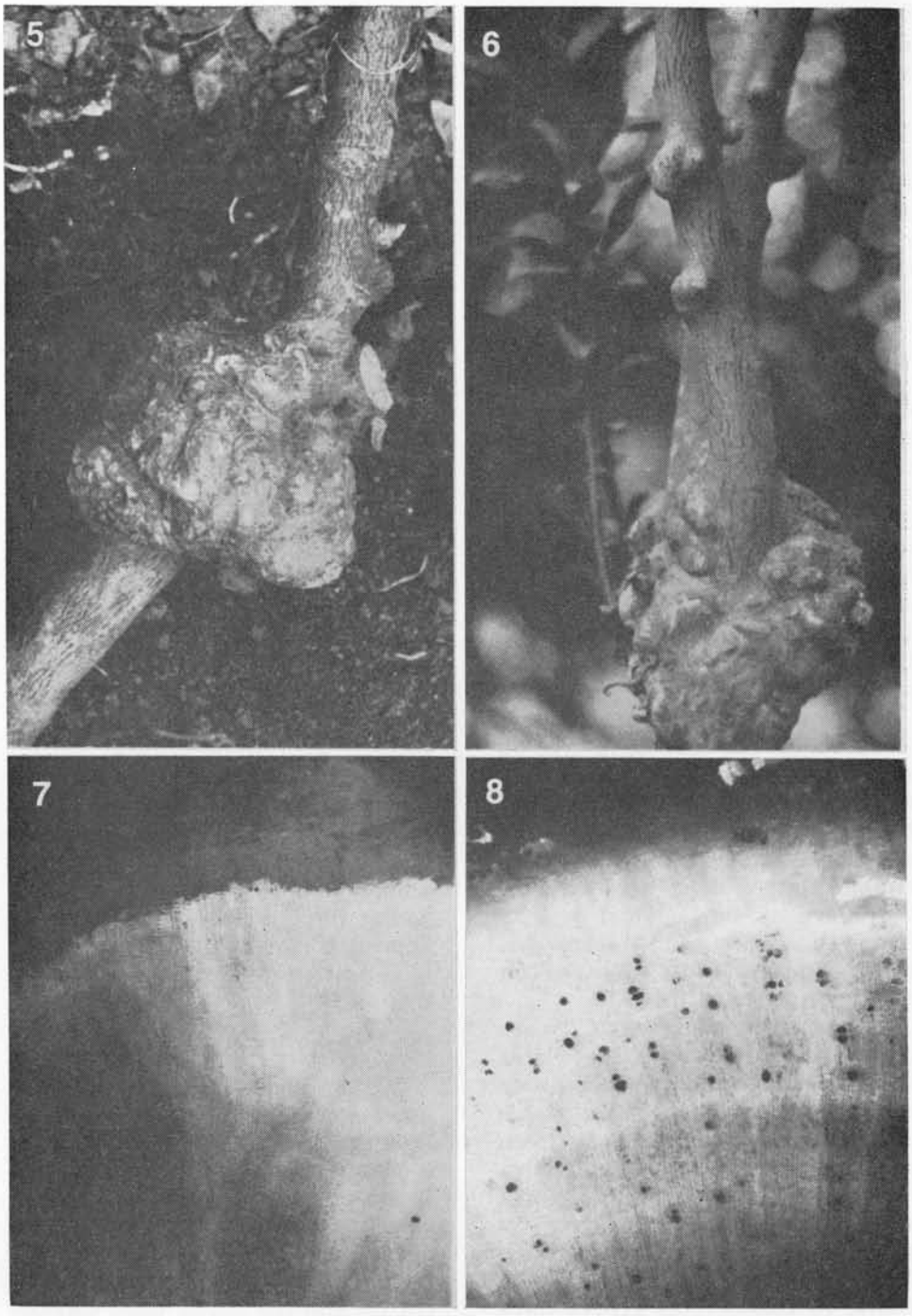

Fig. 5. Extraordinary overgrowth of the budunion in a 15-year-old plant obtained by budding a knotted bud of bergamot on a sour orange seedling.

Fig. 6. Tumor-like formations on stems obtained by topworking a bud of bergamot on an old tree.

Fig. 7. Cross section of a stem of bergamot affected by bud knot showing abnormal growth of xylem, lack of vessels and disorganized woody elements.

Fig. 8. Normal growth of xylem in the opposite side of an affected bud.

repeatedly under tap water. Tissue was disinfected first with 70% ethanol for 5 minutes and then with 2% sodium hypochlorite containing 0.1% Tween 20 for 10 minutes. The sticks were washed with sterilized distilled water several times. Under aseptic conditions, the nodes and internodes (~1 cm long) were cut longitudinally and put on a modified, commercially prepared culture medium. Fifty g/liter sucrose, 100 mg/liter inositol, 0.2 mg/liter thiamine hydrochloride, 1 mg/liter pyridoxine hydrochloride, 1 mg/liter NAA (naphtalene acetic acid), 0.1 mg/liter N^6BA (N^6 benzyl adenine), and 2.5 g gelrite were added to the Murashige and Skoog salts solution (7).

The medium was melted in a microwave oven for 3 minutes and dispensed in test tubes (25 ml each). The test tubes were closed with belko-caps and sterilized by autoclaving for 20 minutes at 121 C. The medium in the test tubes was solidified as slants.

Internodes and nodes were put on the medium and placed in a culture room at 25 ± 2C and 60% relative humidity under 1,000 lux.

Thirty nodes and internodes of bergamot produced a large amount of unorganized callus, more representative of internodes; none produced shoots or roots.

All 10 citron nodes produced large roots and 20% of them also developed shoots.

DISCUSSION

Stem proliferation is well known in different fruit species such as apple (1), olive (2), and citrus too. These are called sphaeroblasts and originate from the outer liberian layer and are restricted to the bark. No connection exists with the woody tissue. As they enlarge they are extruded and later become detached from the bark.

Olive trees are also characterized by ovules which are hyperplastic formations connected to the xylem. They enlarge with age and also remain on old branches and trunks.

Hyperplastic formations such sphaeroblasts, galls (10) and knots (6) have been reported in citrus. The bud knot of bergamot appears different from sphaeroblasts because their origin is related to the cambial activity; different from woody galls because no reaction has been observed after inoculation of Mexican lime and Volkamer lemon; and different from knots because of their distribution on the stem, the high frequency of occurrence and the propagation.

From the above results the reported disorder is caused by a disorganized abnormal formation of xylem elements, localized near the buds. The lack of vessels appears responsible for the yellowing and corking of the veins and the die back of stems and branches.

The disease appears as a bud propagable, non-transmissible disorder. The perpetuation of some symptoms, such as vein corking and uneven growth, through seeds is a further suggestion of genetic origin as observed in several plant genera. The pathway of the disorder seems to be related to an upset in the balance between growth and regulation of growth which induces an irregular, tumor-like growth. This is confirmed by the autonomous, undifferentiated growth of affected tissues. Since the cambium is not affected, the tumors are connected to vascular bundles and lessening of symptoms on older stems is observed.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. BALDINI, E. and B. MOSSE
   1956. Observations on the origin and development of sphaeroblasts in the apple. J. Hort. Sci. 3: 156-62.
2. BALDINI, E. and F. SCARAMUZZI
   1957. Indagini ed osservazioni sugli sferoblasti dell'olivo. Ann. Sper. Agrar. II: 723-40.
3. CATARA, A., M. DAVINO, and G. MAGNANO DI SAN LIO
   1980. Investigations on a bergamot gummosis in Calabria (Italy), p. 281-85. *In* Proc. 8th Conf. IOCV 1979, IOCV, Riverside.
4. CATARA, A., M. DAVINO, and G. MAGNANO DI SAN LIO
   1983. Further researches on a virus-like gummosis of bergamot, p. 247-249. *In* Proc. 9th Conf. IOCV (this volume).
5. CATARA, A., G. PERROTTA, and G. CUTULI
   1968. Rassegna delle malattie virus-simili degli agrumi osservate in Italia. Riv. Ortoflorofrutt. Ital. 52: 722-48.
6. KNORR, L. C.
   1973. Citrus diseases and disorders. Univ. Florida Press, Gainesville.
7. MURASHIGE, T., and F. SKOOG
   1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-97.
8. NAVARRO, L.
   1981. Effect of citrus exocortis viroid (CEV) on root and callus formation by stem tissue of Etrog Citron (*Citrus medica* L.) cultured *in vitro*. 1981 Proc. Int. Soc. Citriculture Vol. 1: 437-439.
9. TERRANOVA, G. and G. CUTULI
   1975. Lo stato fitosanitario del bergamotto in Calabria. Ann. 1st Sper. Agr., Acireale, 7/8, 175-89.
10. WALLACE, J. M. and R. J. DRAKE
   1960. Woody galls on citrus associated with vein-enation virus infection. Plant Dis. Rep. 44: 580-84.