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Host Range of, and Plant Reaction to, *Subanguina picridis*[1]

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Abstract: The host range of the knapweed nematode, *Subanguina picridis* (Kirjanova) Brzeski, under controlled environmental conditions was extended to include, in addition to Russian knapweed, *Acroptilon repens* (L.) DC., plant species within the Centaureinae, and Carduinae subtribes of the Cynareae tribe of the Asteraceae family. Examination of host response to nematode infection revealed that Russian knapweed was the only highly susceptible host plant. Diffuse knapweed (*Centaurea diffusa* Lam.) was moderately susceptible, and other plants which formed galls were resistant to *S. picridis.*

Key words: *Acroptilon repens* (Russian knapweed), biological control, gall, histopathogenesis, hyperplasia, hypertrophy, nutritive cells, *Subanguina picridis* (knapweed nematode).

Most attempts in biological weed control have involved insects and, to a lesser extent, plant pathogens, with few nematodes being evaluated. The nematode *Orrina phyllobia* (Thorne, 1934) Brzeski, 1981, syn.: *Nothanguina phyllobia* (Thorne, 1934) Thorne, 1961, has potential for control of silverleaf nightshade, *Solanum elaeagnifolium* Cav., in the southwestern United States (11,12). The increase and artificial distribution of *O. phyllobia* follows the inundative approach to biological weed control. Another approach is the classical or inoculative approach whereby an exotic organisms's potential is evaluated for control of an introduced or alien weed species. A leaf and stem gall forming nematode, *Subanguina picridis* (Kirjanova, 1944) Brzeski, 1981, syn.: *Paranguina picridis* (Kirjanova, 1944) Kirjanova and Ivanova, 1968, from the Soviet Union parasitizes Russian knapweed, *Acroptilon repens* (L.) DC., syn.: *Centaurea repens* L., a major noxious weed in much of the United States and Canada. The nematode was reported to be host specific and damaging to its host in the Soviet Union (6,9,10). *Subanguina picridis* galled Russian knapweed plants were obtained from the Soviet Union. As with all other prospective exotic biological weed control agents, the host specificity of *S. picridis* needed to be determined before its release in North America (3). The objective of this research was to examine the host range of the knapweed nematode, *S. picridis.* The biology of *S. picridis,* including its potential as a biological control agent, and an examination of the taxonomy of *S. picridis* are presented elsewhere (14,15).

MATERIALS AND METHODS

Nematode inoculum: Infected host plant material was obtained from O. V. Kovalev of the Soviet Union. The material, which included dried stem and leaf pieces heavily galled by *S. picridis,* was stored at room temperature in a dry state until required. Nematode suspensions were prepared by treating galls for 3 minutes in a 1% sodium hypochlorite solution followed by three washes in sterile distilled water. Surface sterilized galls were cut open and placed in a 100-ml beaker containing 50 ml of distilled water. The nematodes were allowed to egress from galls in continuously aerated water overnight. Nematodes were removed by pipette, suspended in distilled water, and adjusted to the desired concentration.

Plants tested: Plants tested included those related to the known host (Russian knapweed), host plants of nematode species related to *S. picridis,* and plants which have vegetative reproduction similar to the known host. The intensity of testing, greatest on species closely related to the known host, decreased as the test plants were more distantly related (13). Plants were grown in vermiculite in 12.5-cm-d pots and watered with Hoagland's solution (4) modified to 10.5 ppm nitrogen. All of the nitrogen was supplied as ammonium nitrate

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TABLE 1. Plant reaction to *Subanguina picridis*.

Plants tested	Gall formation
Asteraceae (Compositae)	
Tubuliforae	
Cynareae	
Centaureinae	
Acroptilon repens (L.) DC. (Saskatchewan)	+
Acroptilon repens (L.) DC. (British Columbia)	+
Acroptilon repens (L.) DC. (Soviet Union)	+
Centaurea diffusa Lam.	+
Centaurea maculosa Lam.	+
Centaurea ×*pratensis* Thuill.	+
Centaurea montana L.	−
Cyanus segetum (L.) Hill	−
Carthamus tinctorius L.	−
Carduinae	
Arctium minus (Hill) Berhn.	−
Carduus nutans L.	+
Cirsium arvense (L.) Scop.	−
Cirsium flodmanii (Rydb.) Arthur	+
Cirsium oleracum (L.) Scop.	−
Cirsium vulgare (Savi) Ten.	−
Cynara scolymus L.	+
Onopordum acanthium L.	+
Carlininae	
Carlina vulgaris L.	−
Echinopinae	
Echinops ritro L.	+
Anthemideae	
Achillea millefolium L.	−
Artemissia gnaphalodes Nutt.	−
Chrysanthemum sp.	−
Arctoteae	
Arctotis acaulis L.	−
Gazania rigens R. Br.	−
Astereae	
Aster sp.	−
Solidago sp.	−
Calenduleae	
Calendula sp.	−
Eupatorieae	
Liatris ligulistylis (A. Nels.) K. Schum.	−
Helenieae	
Gaillardia sp.	−
Tagetes sp.	−
Heliantheae	
Ambrosia psilostachya DC. var. *coronopifolia* (T. & G.) Tarw.	−
Helianthus annuus L.	−
Iva axillaris Pursh	−
Rudbeckia sp.	−
Inuleae	
Antennaria dioica Gaertn.	−
Mutisieae	
Gerbera jamesonii Bolus.	+
Senecioneae	
Senecio jacobaea L.	−
Vernonieae	
Stokesia laevis Greene	−
Liguiflorae	
Cichorieae	
Sonchus arvensis L.	−
Taraxacum officinale Weber	−
Boraginaceae	
Cynoglossum officinale L.	−
Caryophyllaceae	
Silene cucubalis Wibel	−
Chenopodiaceae	
Beta vulgaris L.	−
Convolvulaceae	
Convolvulus arvensis L.	−
Crassulaceae	
Sedum sp.	−
Brassicaceae (Cruciferae)	
Brassica napus L.	−
Thlaspi arvense L.	−
Euphorbiaceae	
Euphorbia esula L.	−
Poaceae (Gramineae)	
Agropyron repens (L.) Beauv.	−
Avena sativa L.	−
Hordeum vulgare L.	−
Secale cereale L.	−
Triticum aestivum L.	−
Zea mays L.	−
Hypericaceae	
Hypericum perforatum L.	−
Fabaceae (Leguminosae)	
Medicago sativa L.	−
Linaceae	
Linum usitatissimum L.	−
Malvaceae	
Gossypium herbaceum L.	−
Polygonaceae	
Fagopyrum esculentum Moench	−
Rosaceae	
Potentilla recta L.	−
Scrophulariaceae	
Linaria vulgaris Mill.	−
Solanaceae	
Lycopersicon esculentum Mill. var. *commune* Bailey	−
Apiaceae (Umbelliferae)	
Daucus carota L.	−

TABLE 2. Nematode reproduction and pathogenic reaction of plants galled by *Subanguina picridis.*

Plant species	Reproduction within galls	Degree of damage*	Host ratings†
Acroptilon repens	+	+ to ++++	Highly susceptible
Centaurea diffusa	+	+ to ++	Moderately susceptible
Centaurea maculosa	+	− to +	Moderately resistant
Centaurea ×pratensis	+	− to +	Moderately resistant
Carduus nutans	+	− to +	Moderately resistant
Cirsium flodmanii	+	− to +	Moderately resistant
Cynara scolymus	+	− to +	Moderately resistant
Onopordum acanthium	+	− to +	Moderately resistant
Echinops ritro	−	− to +	Resistant
Gerbera jamesonii	−	− to +	Resistant

* Degree of damage: − = no visible damage; + = few, small galls; ++ = majority of leaves with small galls; +++ = large galls, some visible distortion of plant; ++++ = numerous large galls, plant severely distorted.

† Host rating: resistant = few small galls, with no reproduction; moderately resistant = few to many galls with limited reproduction, but with no to very slight damage to the host; moderately susceptible = many galls with reproduction and some damage to the host; highly susceptible = numerous galls, with high rate of reproduction and severe damage to the host.

and the iron in a chelate. Solutions were adjusted to pH 6.0 with potassium hydroxide. The potting medium was saturated with nutrient solution every second day and on alternate days saturated with distilled water. All experiments were conducted in plant growth chambers at a 12-hour day with light intensity of 1,600–2,400 ft-c at 15 ± 1 C and a 12-hour night at 10 ± 1C.

Inoculation procedure: Each pot was infested with 1,200–1,500 nematodes suspended in 1 ml of distilled water. The nematode suspensions were pipetted onto the surface of the potting medium. Perennial test species, including five Russian knapweed control plants in each test, were cut back to ground level just before infestation with nematodes. Seeds of annual test species were sown 25 days after nematode infestation. Each plant species was replicated at least five times (one plant per pot). Pots were placed in a randomized complete block design in the growth chambers.

Additional tests were conducted on *Cyanus segetum* (L.) Hill syn., *Centaurea cyanus* L. (bachelor's button), an annual closely related to Russian knapweed. Five seeds were sown (1 cm deep) in moist vermiculite in one pot each at 11, 16, 20, 24, and 33 days after nematode infestation.

Galls 20–40 days old were collected. Some were opened, and the contained nematodes and eggs were fixed in TAF (5). The galls were then fixed, as were intact galls, in formalin–acetic acid–alcohol (8). Fixed galls were washed overnight with running water, dehydrated in a tertiary butyl alcohol series, and embedded in paraffin (7,8). Galls were sectioned at 8 μm thick with a rotary microtome and mounted on glass slides using Haupt's adhesive (7). Most sections were stained with safranin and fast green (2), but at least one slide of each gall was stained with haematoxylin, safranin, and fast green (7) and one was stained with periodic acid–Schiff's reagent and counter stained with fast green (7). Noninfected leaves of the different hosts were processed similarly.

RESULTS

Host range: In growth chambers *S. picridis* induced galls on Russian knapweed and on *Centaurea diffusa* Lam., *C. maculosa* Lam., *C. ×pratensis* Thuill., *Carduus nutans* L., *Cirsium flodmanii* (Rydb.) Arthur, *Cynara*

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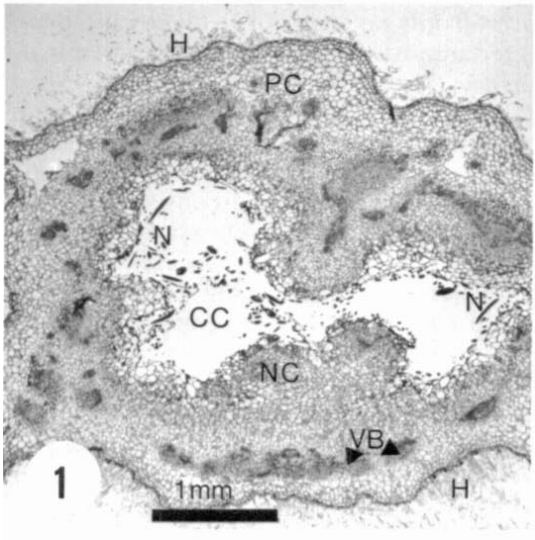
FIGS. 1–6. Cross sections of galls induced on *Acroptilon repens, Centaurea diffusa,* and *Centaurea ×pratensis* by *Subanguina picridis.* (CC = central cavity, H = hairs, IN = cellular inclusions, N = nematodes, NC = nutritive cells, NEC = necrosis, PC = parenchyma cells, VB = vascular bundles.) 1) Cross section of a gall induced on *Acroptilon repens.* 2) Close-up of nutritive cell zone of gall on *Acroptilon repens.* 3) Cross section of a gall induced on *Centaurea diffusa.* 4) Close-up of nutritive cell zone of gall in *Centaurea diffusa.* 5) Cross section of a gall induced on *Centaurea ×pratensis.* 6) Close-up of necrotic layer and cellular inclusions of gall on *Centaurea ×pratensis.*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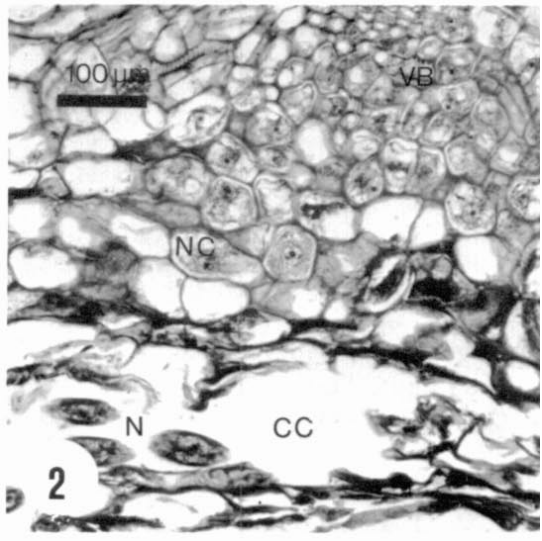
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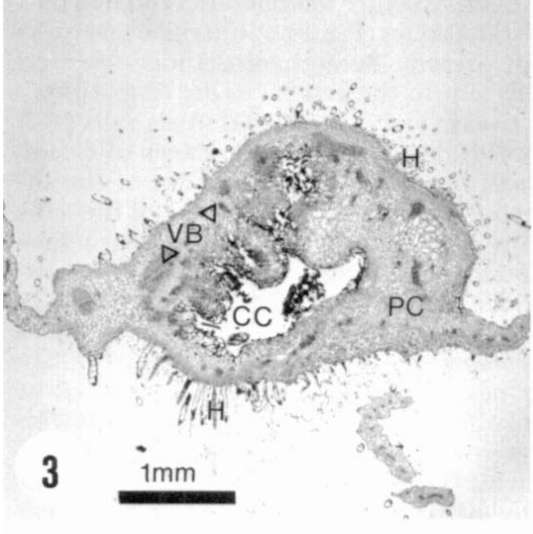
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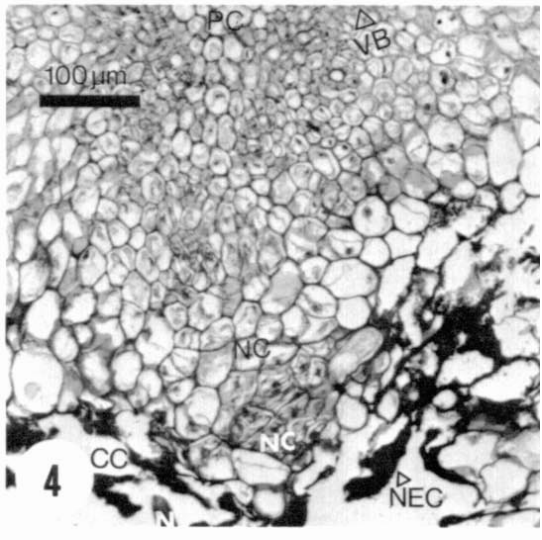
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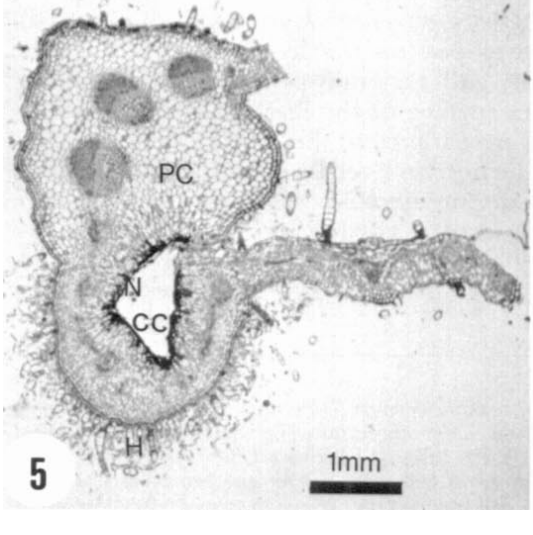
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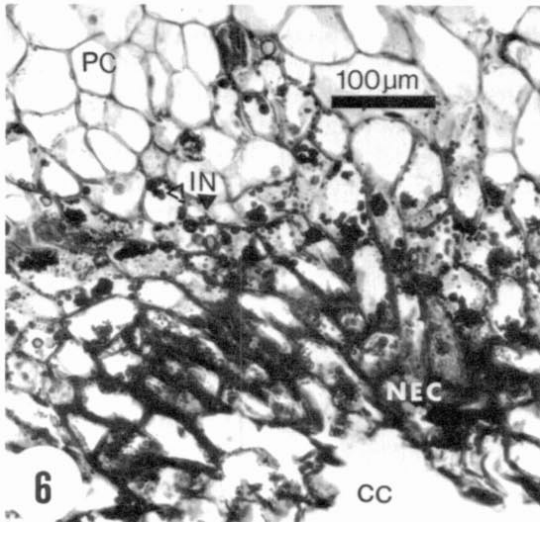
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scolymus L., *Onopordum acanthium* L., *Echinops ritro* L., and *Gerbera jamesonii* (Bolus. (Table 1). Eggs were observed in dissected galls from all the above plants with the exception of *E. ritro* and *G. jamesonii* (Table 2). Twenty-five seedlings of the annual *Cyanus segetum* exposed to approximately 75,000 nematodes failed to produce galls.

Acroptilon repens: The structure and histopathogenesis of *S. picridis* galls on Russian knapweed are described in detail elsewhere (15). For comparison purposes, Figures 1 and 2 illustrate the typical highly susceptible host response of Russian knapweed.

Centaurea diffusa: Galls (2–8 mm d) developed on the rachis, leaflets, and stems of *C. diffusa,* often causing twisting and distortion of infected plants. Galls were not as differentiated into layers as those on Russian knapweed. The central cavity was often convoluted (Fig. 3). Nematodes were occasionally seen beyond the nutritive cell zone feeding in the parenchyma cell zone outside the vascular bundles. A smaller nutritive cell zone was present in these galls. Some necrosis was observed surrounding the central cavity (Fig. 4). The outer layers of the galls were similar to those on Russian knapweed.

Centaurea maculosa: Few, small galls (2–4 mm d) formed on leaves of *C. maculosa* but are not shown here. Few nutritive cells were present, and the gall tissue was primarily composed of enlarged vacuolated parenchyma cells.

Centaurea × *pratensis:* Small galls (1–4 mm d) developed on the petiole, leaf blade, and mid-vein of *C.* × *pratensis.* A wide, dark staining, necrotic layer was adjacent to the central cavity (Figs. 5, 6). Nutritive cells were absent from the galls which consisted largely of enlarged, vacuolated parenchyma cells. Numerous inclusions were evident in cells adjacent to the necrotic layer (Fig. 6).

Carduus nutans: Small galls (3–5 mm d) formed only on leaves of *C. nutans* and projected almost entirely from the lower leaf surface. The cells of the galls were not differentiated into definite layers (Fig. 7); the outer region consisted of elongated palisade-like cells with numerous chloroplasts (Fig. 8). Most of the gall consisted of enlarged, vacuolated, closely packed parenchyma cells with a few vascular bundles partly surrounding the central cavity (Fig. 7). Nutritive cells were not present. Some necrosis and debris were observed adjacent to the central cavity.

Cirsium flodmanii: Only two small galls (3 mm d) developed on leaves of *C. flodmanii.* Similar to most other hosts, hair development was more pronounced on the gall epidermis than on the normal leaf, but the gall structure was not differentiated into distinct zones (Fig. 9). A nutritive layer was not present. Some necrosis was observed adjacent to the central cavity (Fig. 10).

Cynara scolymus: Three small galls (4–5 mm d) developed on the leaves of *C. scolymus* causing minor distortion of the infected leaf. The cellular layers of the galls were not well differentiated (Fig. 11). Few nutritive cells were evident in some regions of the gall (Fig. 12) but were not nearly as extensive as in the Russian knapweed and *Centaurea diffusa* galls. Most of the cell layer next to the central cavity consisted of enlarged, vacuolated parenchyma cells with necrosis occurring adjacent to the central cavity. The outer layers of the gall resembled palisade mesophyll of the normal leaf and often had numerous chloroplasts.

Onopordum acanthium: Galls (3–5 mm d) developed on the leaves of *O. acanthium* with galls generally projecting from the upper surface of the leaf. Galls were similar in appearance to those on *Cynara scolymus.* The nutritive cell layer was poorly defined, and some necrosis was present adjacent to the central cavity (Figs. 13, 14). The parenchyma cell layer extended out to the epidermis of the gall. Cells near the epi-

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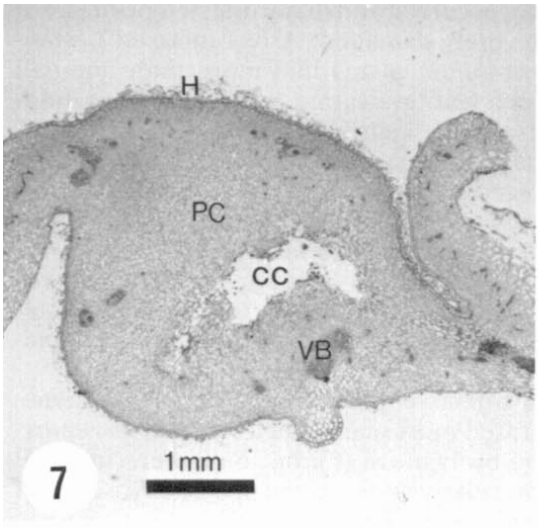
FIGS. 7–12. Cross sections of galls induced on *Carduus nutans, Cirsium flodmanii,* and *Cynara scolymus* by *Subanguina picridis.* (CC = central cavity, CH = chloroplasts, EP = epidermis, H = hairs, N = nematodes, NC = nutritive cells, NEC = necrosis, PC = parenchyma, VB = vascular bundles.) 7) Cross section of a gall induced on *Carduus nutans.* 8) Close-up of layer of parenchyma cells with numerous chloroplasts beneath epidermis of gall on *Carduus nutans.* 9) Cross section of a gall induced on *Cirsium flodmanii.* 10) Close-up of cell types from central cavity to epidermis of gall on *Cirsium flodmanii.* 11) Cross section of gall induced on *Cynara scolymus.* 12) Close-up of cell types from central cavity to epidermis of gall on *Cynara scolymus.*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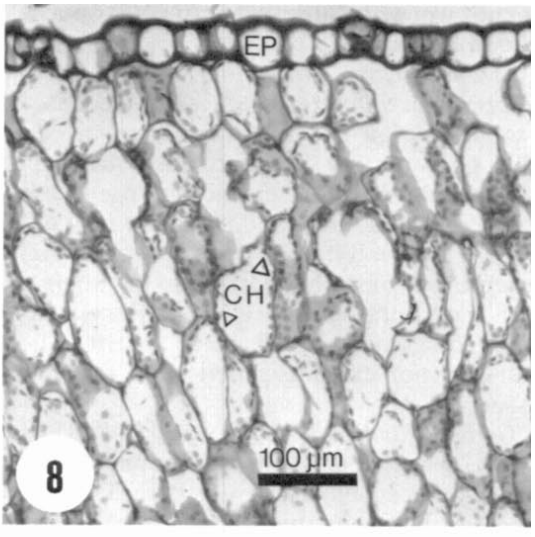
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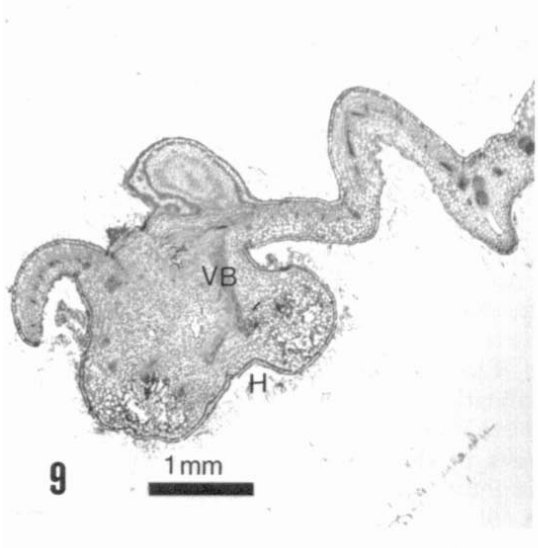
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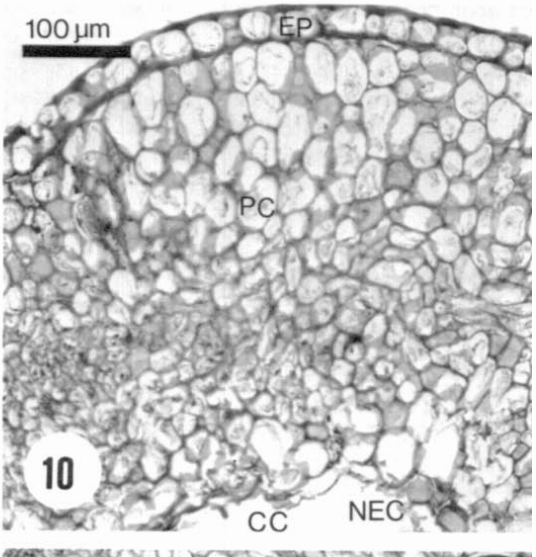
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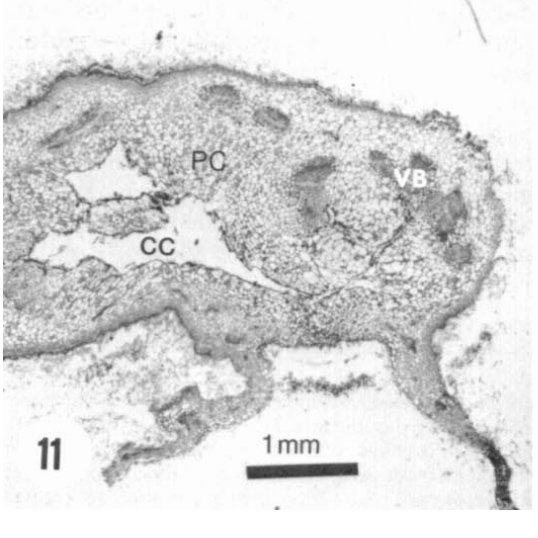
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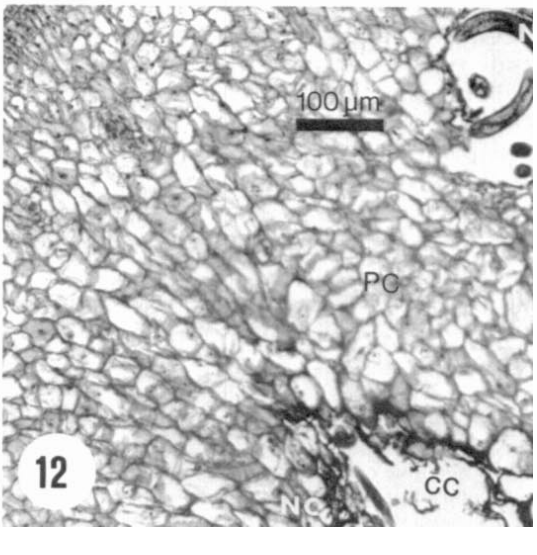
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dermis were not supplied with chloroplasts (Fig. 14).

Echinops ritro: Only one small gall developed on *E. ritro* which appeared as a whitish region on the midvein of a leaf. The gall was not differentiated into cell layers and was almost entirely composed of closely packed, enlarged parenchyma cells (Figs. 15, 16). The outer layers of the gall resembled mesophyll cells of the normal leaf and contained numerous chloroplasts (Fig. 16). Nutritive cells were absent. The gall was not circumscribed with vascular tissue.

Gerbera jamesonii: Four small galls (2–4 mm d) developed on one *G. jamesonii* plant. The galls caused minor distortion of the infected leaf, but nutritive cells were not present. Extensive necrosis occurred adjacent to the small central cavity (Figs. 17, 18). The gall consisted almost entirely of enlarged, vacuolated parenchyma cells with the vascular bundles laterally displaced around the central cavity.

Discussion

The host range of *S. picridis* includes Russian knapweed and a few closely related species in the Centaureinae and Carduinae subtribes of the Cynareae tribe of the Asteraceae family. Galls were induced and reproduction of *S. picridis* occurred in plants of seven species in these two subtribes. Galls also formed on *Echinops ritro* and *Gerbera jamesonii,* but the nematode failed to reproduce, so they cannot be considered hosts of *S. picridis.*

A host rating scheme was utilized in this study to differentiate between susceptible and resistant host responses to *S. picridis.* Resistant plants were characterized by good host growth and poor parasite reproduction, whereas susceptible plants were characterized by poor host growth and good parasite reproduction (1). In these studies, Russian knapweed was the only plant highly susceptible to *S. picridis.* Extensive galling occurred and many infected plants were severely damaged. A few infected *Centaurea diffusa* plants had more than one gall on a leaf, indicating that *C. diffusa* is moderately susceptible to the nematode. Slight damage occurred on the other hosts, indicating tolerance to the parasite. *Echinops ritro* and *Gerbera jamesonii* were galled but resistant, as the nematode failed to reproduce. All other plant species tested were immune, as no galls were initiated, although most were penetrated by the nematodes.

Infective juveniles of *S. picridis* penetrated most plant species tested, as reported by Ivanova (6), but galls were induced on relatively few plant species. Absence of gall formation in some of the Cynareae tribe species may be due to different types of development and growth patterns of the young shoots of these plants. Two annual species, *Cyanus segetum* and *Carthamus tinctorius* L. (safflower), which are closely related to Russian knapweed, did not develop galls when inoculated with *S. picridis.* Even when *C. segetum* was seeded at different times after adding nematodes to pots to ensure suitable meristematic tissue for infective nematodes to penetrate, no galls were formed.

The histological data support the classification of the plant species galled by *S. picridis* as susceptible or resistant to the attack of the nematode. Russian knapweed is definitely the best host that is known for *S. picridis,* and plants were severely damaged by the nematode. The nematode induced elaborate galls well supplied with nutritive cells, and nematode reproduction was copious. Nutritive cells were reduced or absent in galls in other plants when compared to the Russian knapweed galls. Extensive necrosis developed in some of the galls, producing a barrier between the developing nematodes and the nutritive cells or feeding regions of the galls. Although juveniles and eggs in the galls were not

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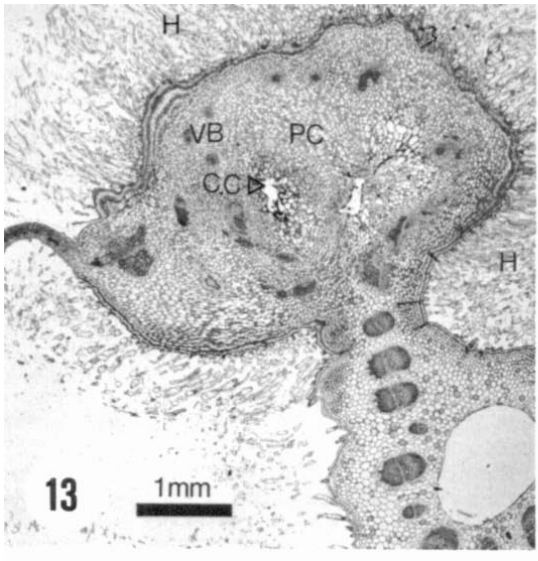
Figs. 13–18. Cross section of galls induced on *Onopordum acanthium, Echinops ritro,* and *Gerbera jamesonii* by *Subanguina picridis.* (CC = central cavity, CH = chlorolasts, EP = epidermis, H = hairs, N = nematodes, NC = nutritive cells, NEC = necrosis, PC = parenchyma cells, VB = vascular bundles.) 13) Cross section of gall induced on *Onopordum acanthium.* 14) Close-up of cell types from central cavity to epidermis of gall on *Onopordum acanthium.* 15) Cross section of gall induced on *Echinops ritro.* 16) Close-up of cell types from central cavity to epidermis of gall on *Echinops ritro.* 17) Cross section of gall induced on *Gerbera jamesonii.* 18) Close-up of cell types from central cavity to epidermis of gall on *Gerbera jamesonii.*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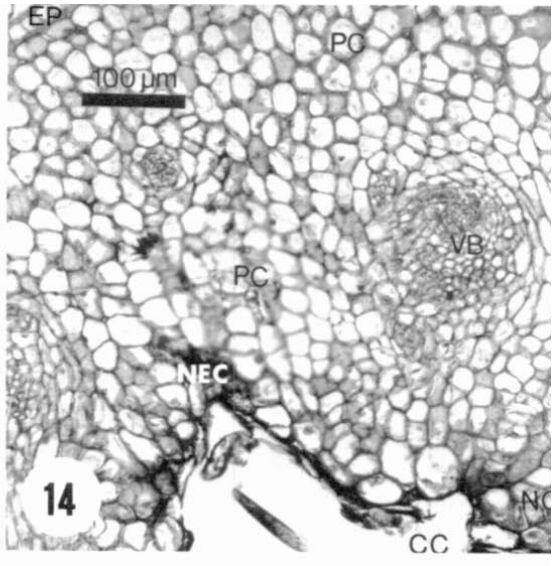
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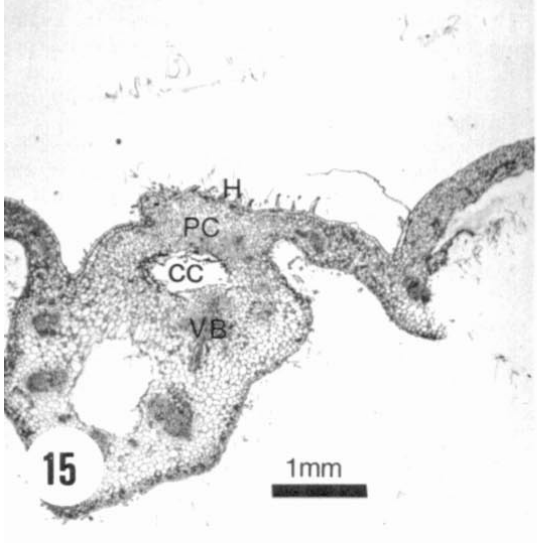
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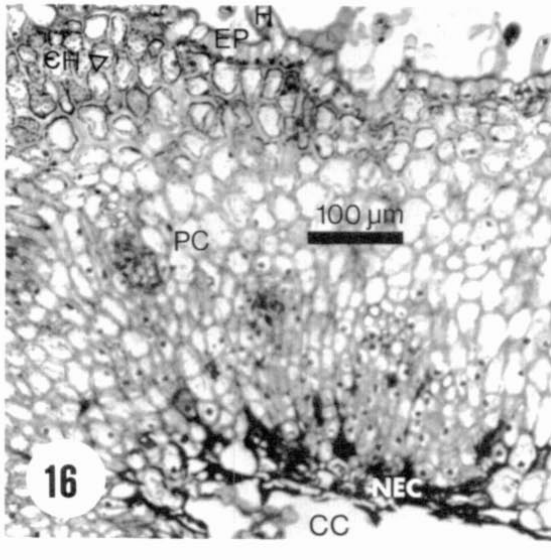
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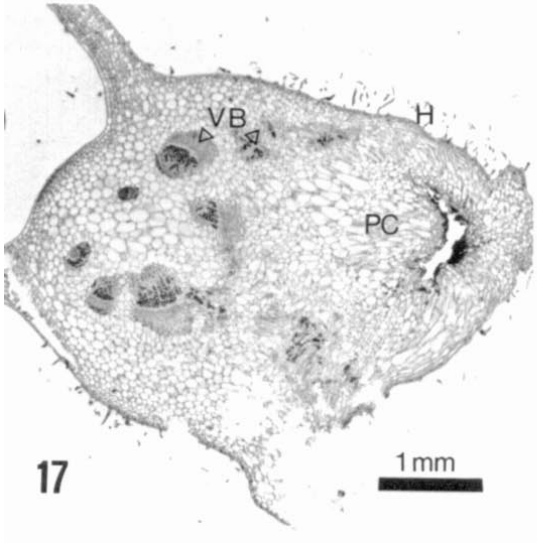
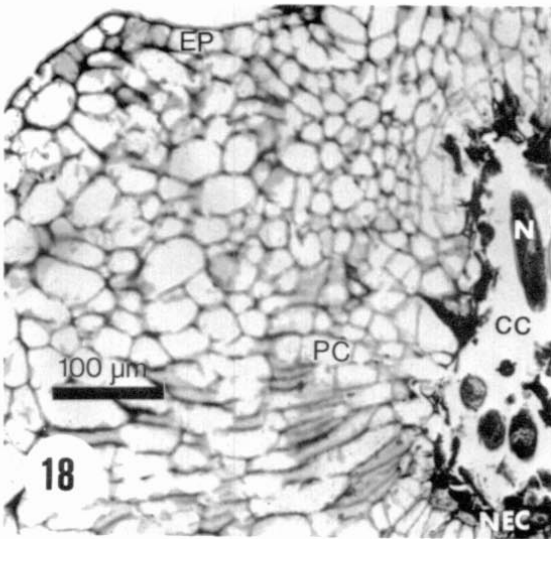
NEC
CC


VB
H
PC
17
1mm


EP
N
CC
PC
100 µm
18
NEC

counted, galled plants other than Russian knapweed supported low or no nematode reproduction.

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