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Biology of *Subanguina picridis*, a Potential Biological Control Agent of Russian Knapweed[1]

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Abstract: The knapweed nematode, *Subanguina picridis*, forms galls on the leaves, stems, and root collar of Russian knapweed, *Acroptilon repens*. After being revived from a dormant, cryptobiotic state, second-stage juveniles required at least 1 month in a free-living state before becoming infective. Galls were induced on relatively slow-growing host plants that retained their apical meristems at or near the soil surface for 2–5 weeks. Galls developed extensive areas of nutritive tissue. The nematode was introduced from the Soviet Union and released in Canada for the biological control of Russian knapweed.

Key words: *Acroptilon repens* (Russian knapweed), biological weed control, gall, histopathogenesis, host specificity, physiological sink, *Subanguina picridis* (knapweed nematode).

Russian knapweed (*Acroptilon repens* (L.) DC., syn.: *Centaurea repens* L.) is a persistent, deep-rooted, perennial weed species introduced into North America in the early 1900s as a contaminant of Turkistan alfalfa (3). It is widely distributed in the western and central regions of Canada and the United States. Its biology has been reviewed (18). Control of Russian knapweed by cultural and chemical methods is difficult and not satisfactory for most infestations.

Russian knapweed has few natural enemies in North America, but in its native range it is attacked by numerous specialized organisms including a leaf and stem gall nematode, *Subanguina picridis* (Kirjanova, 1944) Brzeski, 1981. Populations of Russian knapweed in the southern Soviet Union are parasitized by the nematode, which induces galls on the stems, leaves, and root collars causing reduction in plant growth and seed production (8,14). The nematode was reported to be host specific (8,11) and has been artificially spread, as crushed gall material or in a water suspension, for control of Russian knapweed in the Soviet Union (12,13).

Plant material heavily infected with nematode galls was imported into Canada and studied in the quarantine research facilities at the Agriculture Canada Research Station in Regina, Saskatchewan, to determine the potential of the knapweed nema-

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tode as a biological control agent of Russian knapweed in North America. The objectives of the program were to 1) develop a laboratory system for propagating the nematode, 2) study the biology of the nematode under controlled environmental conditions, 3) verify the reported host specificity, and 4) evaluate its potential to control Russian knapweed. Some of the work with *S. picridis* is reported in two companion papers (19,20).

Materials and Methods

Nematode inoculum was prepared by treating dried stem and leaf galls for 3 minutes in a freshly prepared 1% sodium hypochlorite solution followed by three washes in sterile distilled water. Galls were dissected and immersed in sterile distilled water with continuous aeration overnight. Motile nematodes were removed by pipet, suspended in sterile distilled water, and used immediately as inoculum. Three experiments were conducted to determine optimum conditions for gall formation on the host, Russian knapweed.

Experiment 1: Shoots (10–15 cm tall) of knapweed plants growing in soil were infested with approximately 1,000 nematodes suspended in 1 ml distilled water with a pipet. The inoculated shoots were immediately wrapped in moistened filter paper and covered with plastic bags (15) for 4 days. After removing the bags and filter paper, one pot containing 5–8 inoculated shoots was placed in the following environments: 1) 14 hours daylight, minimum of 800 ft-c, 22 ± 5 C; 2) dark at 10 C for 4 days, then 14 hours daylight, minimum of 800 ft-c, 22 ± 5 C; 3) 10 C with 10 hours daylight (400 ft-c); and 4) 8.5 hours light (800 ft-c) at 22 ± 5 C, 7.5 hours light (400 ft-c) at 10 C and 8 hours dark at 10 C. Six days after inoculation two shoots from each treatment were removed, stained with cotton blue lactophenol (6), and examined for nematode penetration. The experiment was terminated after 30 days.

Experiment 2: Soil of six 20-cm-d pots containing Russian knapweed was inoculated with crushed galls at 100 g/m² of soil surface. Inoculated pots were kept in 14-hour photoperiod, minimum of 800 ft-c, and at 22 ± 5 C. The experiment was terminated after 60 days.

Experiment 3: Russian knapweed plants were grown from root pieces in vermiculite in 12.5-cm-d pots. The vermiculite soil medium was saturated with modified Hoagland's solution (5) every second day and on alternate days with distilled water. Four levels of nitrogen as ammonium nitrate were applied: 2.1 ppm, 10.5 ppm, 210 ppm (standard Hoagland's solution), and 420 ppm. Chelated iron was supplied, and solutions were adjusted to pH 6.0 with potassium hydroxide. Ten replications of the four treatments were placed in a randomized complete block design in a plant growth chamber under the following conditions: day temperature 15 ± 1 C; day length, 12 hours; light intensity, 1,000 ft-c; night temperature, 10 ± 1 C. After 6 weeks shoots were removed from all pots and approximately 2,000 nematodes suspended in 1 ml of distilled water were applied to the vermiculite surface.

Histopathogenesis of Russian knapweed galls: Nematode-induced galls were collected, cut open, and fixed in FAA (10) on days 1, 4, 7, 14, 21, 28, and 40 after their appearance. Fixed tissues were washed in running water overnight, dehydrated in a tertiary butyl alcohol series, and embedded in paraffin (9,10). All tissues were sectioned at 8 μm with a rotary microtome and mounted on glass slides using Haupt's adhesive (9). Sections were stained with safranin and fast green (4), with at least one slide of each gall stained with haematoxylin, safranin, and fast green (9) and one with periodic acid-Schiff's reagent and counterstained with fast green (9). A total of 21 galls were examined.

Results

Experiments 1–3: Numerous galls developed on the leaves and stems of Russian knapweed once the appropriate techniques were developed and proper environmental conditions found. Although nematodes penetrated shoots under all four environmental conditions studied in experiment 1, only two small galls formed at 10 C with a 10-hour photoperiod. No galls formed in experiment 2, but consistent gall formation was achieved in experiment 3. Galls formed on plants in all 10 replicates of the 10.5-ppm N treatment, but few galls formed on plants at the other N levels.

Galls became visible 38–84 days after inoculation.

Histopathogenesis of Russian knapweed galls: Galls on stems often visibly distorted plant growth. Galls on the leaves (Fig. 1) were 5–12 mm d, compared with the average 0.25-mm thickness of the normal leaf (Fig. 2). Nematodes were contained in a central cavity in the gall which was surrounded by a layer of large vacuolated cells interspersed with debris from collapsed cells (Figs. 3, 4). The contents of these vacuolated cells probably had been consumed by the feeding nematodes. Outside the inner layer of cells was a densely staining layer of nutritive cells (Figs. 4, 5). These cells were nonvacuolated and contained cytoplasm. The nutritive cells had a large nucleus, often with two or three nucleoli. These cells radiated toward the center of the gall from the vascular bundles which surrounded the nutritive layer. The vascular bundles had been laterally displaced and circumvented the gall. A broad zone of enlarged, tightly packed parenchyma cells was evident between the vascular bundles and the gall epidermis (Fig. 5). These cells were vacuolated and not as densely stained as the cells of the nutritive layer; occasionally they contained chloroplasts in the layers just below the epidermis (Fig. 6). The epidermis consisted of cells slightly larger than those of the normal leaf. Development of hairs on the epidermis of the gall (Fig. 6) was more extensive and the cuticle of the gall thicker than on a normal leaf.

Discussion

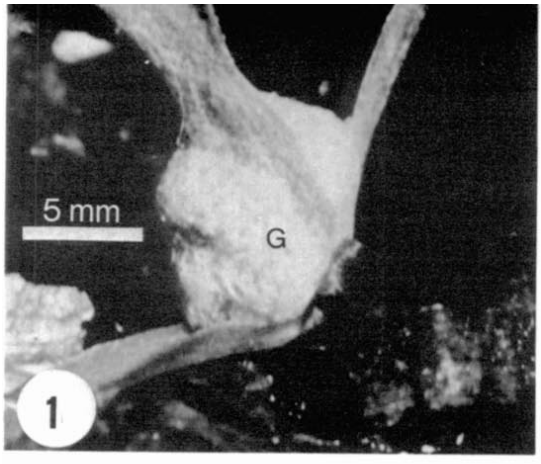
In these studies, consistent gall formation was obtained only when Russian knapweed was grown in a relatively cool, moist, infertile environment and after the nematodes had been in a free-living state for at least 1 month. The importance of the physiological state of the host plant to infection and gall formation by leaf gall forming nematodes has been observed in other studies (17,22). Gall formation is dependent upon the ability of the parasite to disrupt the host's normal growth pattern and to redirect the host to form a gall through extensive hyperplasia and hypertrophy. Therefore, suitable undifferentiated meristematic tissue must be available for gall formation to be induced. *Subanguina picridis* galls were induced only under conditions where vegetative root buds were maintained at or just beneath the soil surface and were not undergoing rapid elongation and differentiation. The life cycle of *S. picridis* was described by Ivanova (8).

Subanguina picridis juveniles were unable to induce gall formation on their hosts until a period of time had elapsed after being revived from a dormant state, as Goodey (2) noted for *Anguina millefolii*. Second-stage juveniles of *S. picridis* go into a state of quiescence (cryptobiosis) induced by dehydration when the host plant senesces at the end of the growing season. Metabolism essentially ceases during cryptobiosis (1). *Subanguina picridis* juveniles were easily revived from cryptobiosis by the addition of water but were unable to induce gall formation until at least 1 month after being revived.

Galls induced by *S. picridis* on Russian knapweed have a well-defined zone of numerous nutritive cells. This nutritive cell zone is more substantial than those reported in other leaf galls induced by nematodes (17,22). Since the volume of nutritive cell layer is a likely indication of the extent of the physiological sink effect (22), the large nutritive zone in *S. picridis*-induced galls suggest that this nematode may be very detrimental to its host.

This study represents the first attempt to introduce an exotic plant-parasitic nematode for the biological control of a weed in North America. It also represents the first time that the nematode *S. picridis* has been propagated in the laboratory.

The development of galls and subsequent reproduction on globe artichoke (*Cynara scolymus* L.) in the laboratory studies would have precluded the use of this nematode to control Russian knapweed and possibly *Centaurea diffusa* in North America, but it is unlikely that *S. picridis* will ever become a pest of globe artichoke in North America. In controlled environment studies, 70 globe artichoke plants inoculated with approximately 1,500 nematodes per plant had only one small gall form on each of three plants with little nematode reproduction (19). In addition, the cultivation and cropping practices for globe artichoke in the United States would not favor the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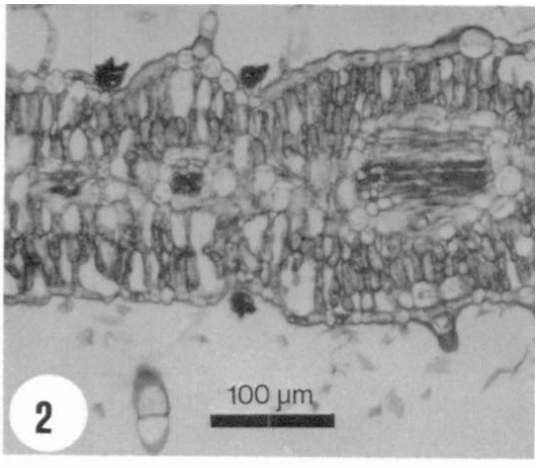
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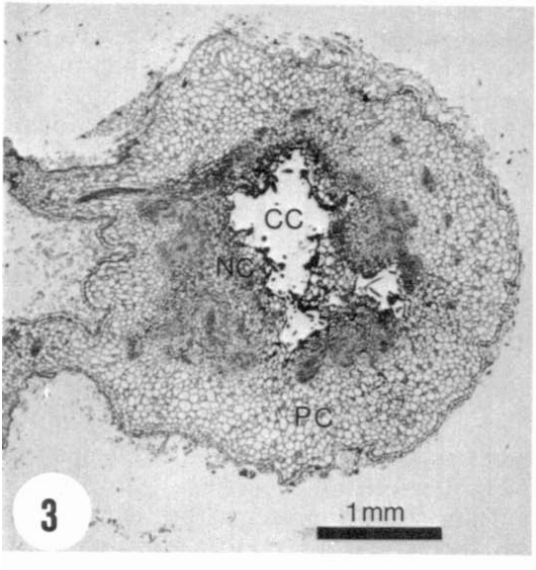
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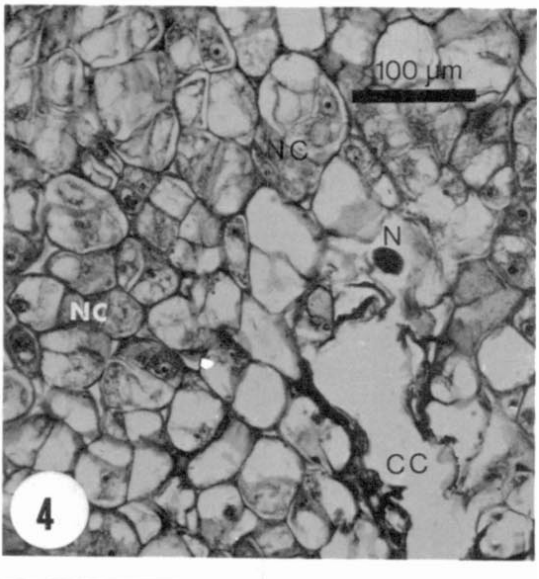
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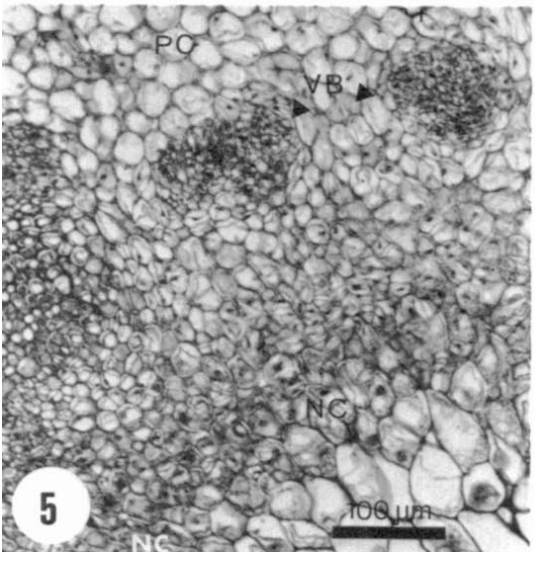
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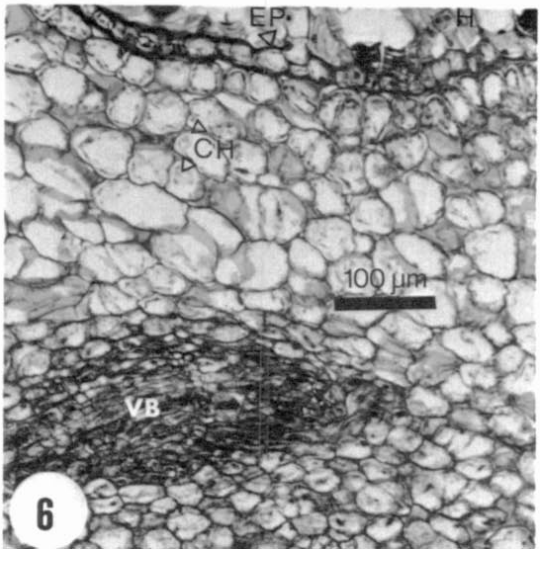
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nematode (16). Heavy nitrogen applications resulting in rapidly growing plants and the wide plant spacing would be detrimental to the nematode. In restricted field trials, no galls were formed on any crop plants, including globe artichoke. The nematode was authorized for release in Canada (21).

In addition to having a restricted host range, a prospective biological weed control agent must be able to survive and flourish in the habitats in which releases are planned. *Subanguina picridis* has become established in three locations in Canada (21). Field populations of the nematode may require time to build up, and artificial dispersal may be necessary. Experiments in the Soviet Union have shown that in plots inoculated with crushed gall material (100 g/m^2), up to 100% of the knapweed was infected and of those plants infected, over 20% were destroyed and over 30% were severely damaged (7,8). Because of its recent introduction, no assessment has yet been made of the effect of *S. picridis* on North American populations of Russian knapweed.

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FIGS. 1–6. Structure and histopathogenesis of *Subanguina picridis* induced leaf galls on *Acroptilon repens* and a noninfected leaf. 1) Surface view of a gall. 2) Cross section of a normal leaf. 3) Cross section of a gall. 4) Cross section of a gall showing the nutritive layer. 5) Cross section of a gall showing vascular bundles between the nutritive layer and the parenchyma layer. 6) Cross section of a gall showing the region from the vascular bundles out to the epidermis. (CC = central cavity, CH = chloroplasts, EP = epidermis, G = gall, H = hairs, N = nematodes, NC = nutritive cells, PC = parenchyma cells, VB = vascular bundles.)

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