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Effects of Temperature, Shoot Age, and Medium on Gall Induction by *Subanguina picridis* in Vitro

X. Ou and A. K. Watson[1]

Abstract: The influence of temperature, shoot age, and medium on gall induction by *Subanguina picridis* on Russian knapweed (*Acroptilon repens*) was examined in vitro. The optimal temperature for gall formation was 20 C. Gall induction was delayed as the temperature decreased, and decreased as shoot age increased. Bud primordia (0-day-old shoots and 5-day-old shoots) with an average length of 4.2 mm and 7.9 mm were the most suitable tissues for nematode development and gall formation. Gall formation was more effective on B5G medium than on MSG. Young shoots under slow growth were most suitable for mass rearing of *S. picridis*.

Key words: Acroptilon repens, culture, gall induction, knapweed nematode, medium, nematode, Russian knapweed, shoot age, *Subanguina picridis*, temperature.

Biological, chemical, and physical factors in the culture system affect the culture of nematodes in vitro. Biological factors include the type and age of the plant tissue. Chemical factors include nutrient status and plant growth regulators in the culture medium. Physical factors include temperature and light intensity. Of these various factors, temperature is likely to be the most important, because it influences the behavior, development, and reproduction of nematodes (1,3,5,13), and the response of plants to nematode infection (10). Penetration, development, and the population density of nematodes are affected by the age of the plant tissue; young tissue is generally more susceptible to nematode infection than older tissue (2). In addition, nematode development is greatly influenced by culture medium. The rates of development and reproduction of nematodes in culture are affected by the concentration of macronutrient salts, vitamins, and plant growth regulators in the culture medium (4,7,9,16).

The plant-parasitic nematode *Subanguina picridis* (Kirjanova) Brzeski has been imported and released in North America for biological control of Russian knapweed (*Acroptilon repens* (L.) DC) (14,15). Prospects of successful biocontrol of Russian knapweed have been enhanced recently by a technique to mass-rear *S. picridis* in vitro (11,12). The objectives of this study were to determine the effects of temperature, shoot age, and medium on the culture of *S. picridis* in vitro in order to maximize nematode development and reproduction.

Materials and Methods

Effect of temperature on gall induction: Four-month-old cultured *S. picridis* galls (12) were cut open in sterile distilled water in a sterile petri dish, and nematode suspensions were obtained. Individual cultured Russian knapweed shoots (11) were carefully separated from clusters in a sterile petri dish using forceps. Three small shoots, ranging in size from 0.3 to 0.5 cm, were inserted into B5G (12) medium in a petri dish (20 × 60 mm). Nematode suspensions, each with 50 nematodes at various stages of development, were applied to the surface of the medium in each petri dish with a pipette.The inoculated shoots, 10 replicates (10 petri dishes) each, were placed in four separate incubators at 10, 15, 20, or 25 C with 16 hours of light at an intensity of 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Gall induction was monitored for 40 days.

Effect of shoot age on gall induction: Individual lateral bud primordia, approximately 4.2-mm long, derived from cultured Russian knapweed shoots, were transferred to MSIBG medium (12). Pri-

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[1] Postdoctoral research assistant and Professor, Department of Plant Science, Macdonald Campus of McGill University, 21,111 Lakeshore Rd., Ste.-Anne-de-Bellevue, Quebec H9X 3V9, Canada.

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mordia were cultured for 0, 5, 10, or 15 days, attaining averaged shoot lengths of 4.2 mm, 7.9 mm, 12.6 mm, and 16.8 mm, respectively. Three shoots of an age (size) were transferred into a petri dish containing B5G medium. Each treatment was made up of 10 replicates (10 petri dishes), with 50 nematodes at various stages of development applied to each petri dish. Infested petri dishes were placed in a completely randomized design in an incubator at 20 C, with 16 hours of light at an intensity of 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Galls were counted over a period of 26 days.

Effect of medium on gall induction: Fifty nematodes at different stages of development, from a 4-month-old gall, were added to petri dishes with three Russian knapweed shoots grown in either MSG medium (Murashige and Skoog's MS medium as modified by Linsmaire and Skoog [8] supplemented with 1.0 $\mu g/ml$ gibberellic acid) or B5G medium. Each treatment was composed of 10 replicates. The infested petri dishes were placed in a completely randomized design in an incubator at 20 C with 16 hours of light at an intensity of 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Gall induction was observed for 26 days.

Data analysis: All experiments were repeated three times, and data were analyzed by regression analysis.

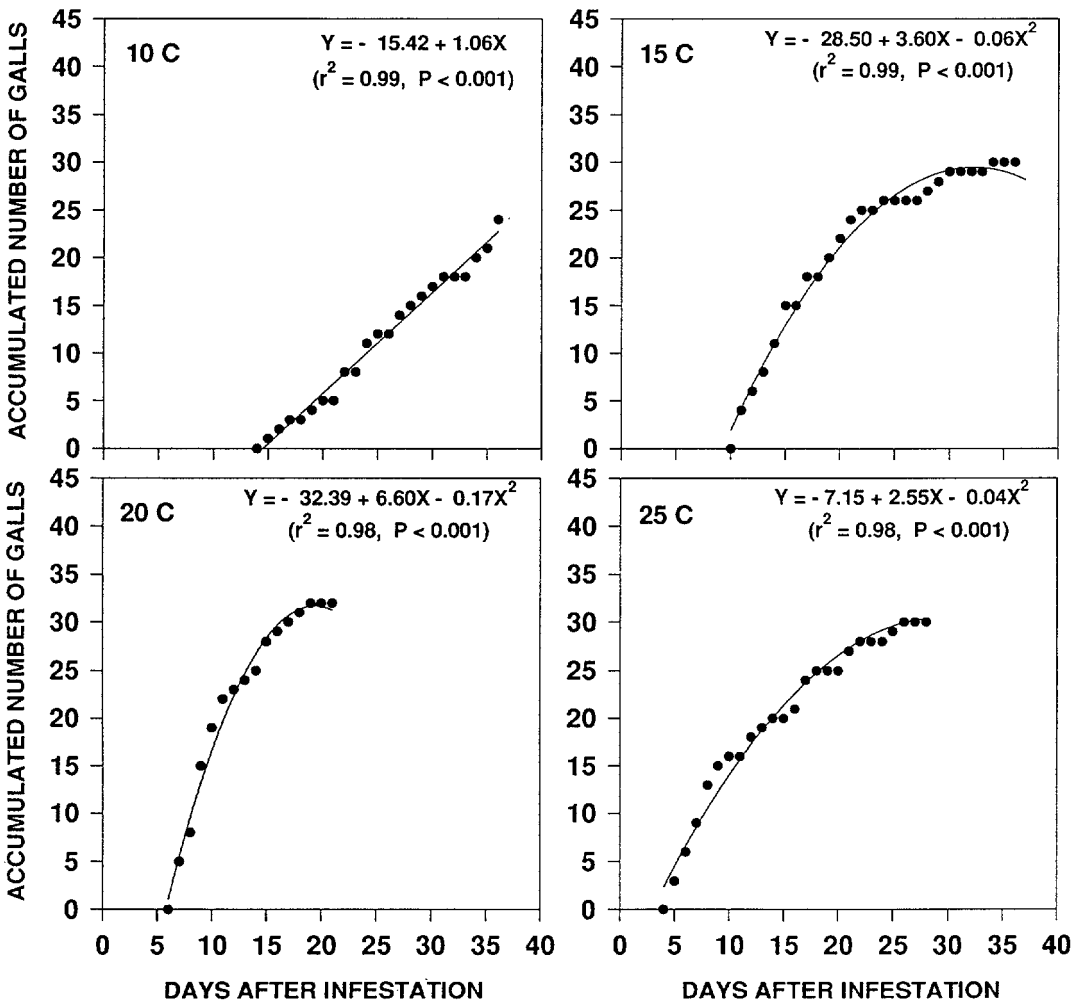

FIG. 1. The effect of four temperatures on *Subanguina picridis* gall induction on Russian knapweed. Gall numbers were accumulated from 10 replicate petri dishes with a total of 30 shoots.

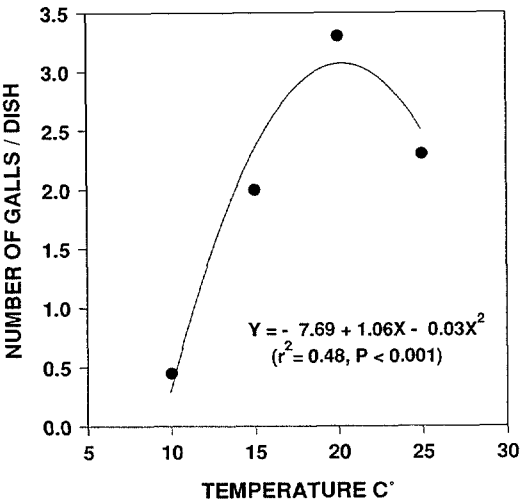

FIG. 2. The effect of temperature on the number of galls induced on Russian knapweed by *Subanguina picridis*. Data presented represent the average number of galls per dish, 19 days after inoculation.

RESULTS

Effect of temperature on gall induction: Gall formation response to culture time varied with temperature. There was a linear relationship between the number of galls and culture time at 10 C (Fig. 1), with the number of galls increasing steadily during 40 days. The relationship between the number of galls and culture time was nonlinear at 15, 20, and 25 C, where the number of galls levelled off after 20 to 25 days in culture. Gall induction time was delayed as the temperature decreased (Fig. 1), with the first galls forming 5 days after inoculation at 25 C, 7 days at 20 C, 9 days at 15 C, and 11 days at 10 C. After 40 days of culturing, the total number of galls in each treatment (30 shoots) was 24, 30, 32, and 31 galls at 10, 15, 20, and 25 C, respectively. The overall frequency of galling (galled shoots/total shoots) was 70% at 10 C, 93% at 15 and 20 C, and 73% at 25 C. Gall induction was nonlinearly related to temperature between 10 and 25 C (Fig. 2). Nineteen days after inoculation, the largest number of galls was formed at 20 C and the fewest number of galls was formed at 10 C.

Effect of shoot age on gall induction: There was a curvilinear relationship between the number of galls and culture time for the 0-day, 5-day, and 10-day shoots infected with *S. picridis* (Fig. 3). The relationship between the number of galls and culture time was linear for 15-day-old shoots. Gall induction time varied with shoot age, with galls appearing 7 days after inoculation on 0-day-old shoots, but not until 9 days after inoculation on 5-day, 10-day, and 15-day shoots. A greater ($P \leq 0.01$) number of galls was produced in young (0-day) shoots than in older shoots. By 26 days after inoculation, the total number of galls in each treatment (30 shoots) was 38, 29, 25, and 15 for 0-day, 5-day, 10-day, and 15-day shoots, respectively. The overall frequency of galling (galled shoots/total shoots) was 90%, 83%, 76%, and 33% for 0-day, 5-day, 10-day, and 15-day shoots, respectively. There was a negative linear relationship between the number of galls and shoot age over the age range of 0 to 15 days (Fig. 4).

Effect of medium on gall induction: The relationship between the number of galls produced and culture time was curvilinear for cultures in B5G or MSG medium (Fig. 5). Although the time to formation of galls was the same (7 days) for shoots cultured on both media, a greater ($P \leq 0.01$) number of galls was produced on B5G (4 galls per shoot) than on MSG medium (2 galls per shoot). Twenty-six days after inoculation, 38 galls were found in the 10 petri dishes of B5G medium, whereas only 21 galls were found on the shoots in MSG medium. The overall gall formation frequency (galled shoots/total shoots) was 90% on B5G medium and 70% on MSG medium.

DISCUSSION

Subanguina picridis depends on host plant suitability for successful establishment, and this suitability may be biochemically oriented or may involve plant growth and developmental patterns (14,15). In the present study, temperature affected gall induction time, the number of galls, and gall formation frequency. Galls formed earlier at 25 C during early stages, ap-

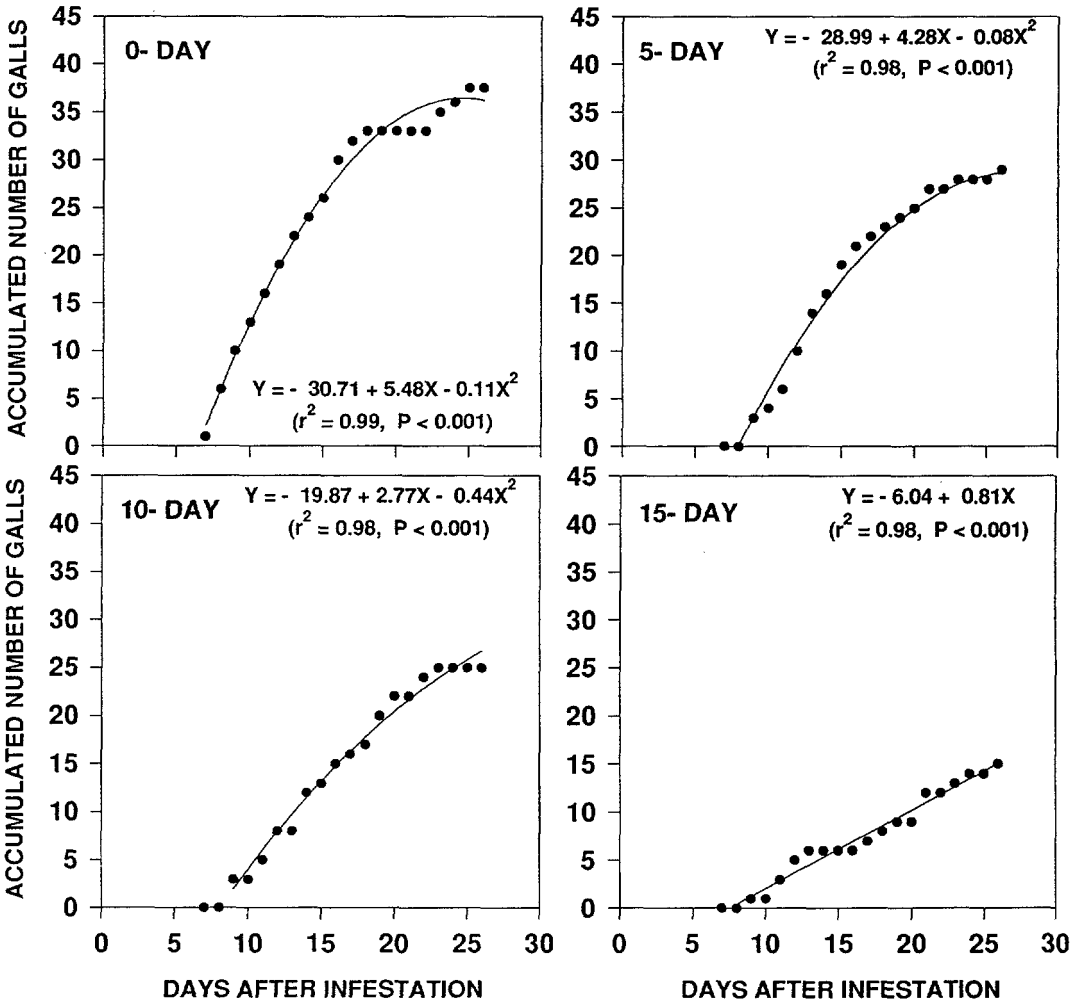

FIG. 3. The effect of Russian knapweed shoot age at inoculation on gall induction by *Subanguina picridis*. Gall numbers were accumulated from 10 replicates with a total of 30 shoots.

peared 2 days later at 20 C, and were further delayed at lower temperature. The final numbers of galls were similar in cultures at 15, 20, and 25 C, whereas a temperature of 10 C induced the fewest galls. The maximum number of galls was obtained in the shortest time at 20 C. These results indicate that of the temperatures tested, 20 C is the optimum temperature for the culturing of this nematode.

Krusberg (6) observed that younger or meristematic cells were more susceptible to modification by nematodes than were older cells, and also that physiologically older tissue, such as cortical parenchyma, exhibited only cell separation rather than galling. The results from the present study showed that the age of the shoot influences the rate of gall formation and the number of galls formed. The most suitable tissues for nematode development and gall formation were 0- and 5-day-old shoots, with an average length of 4.2 and 7.9 mm. The gall formation rate decreased as shoot age increased. Fifteen-day-old shoots with an average length of 16.8 mm were poor tissues for gall formation. The effect of shoot age on gall formation indicates that the young shoots are more susceptible to attack by the nematode; and as the shoot continues to grow, the susceptibility of the host to nematode gall initiation is de-

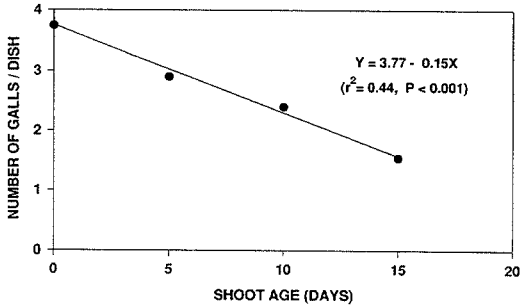

FIG. 4. The effect of shoot age of Russian knapweed on the number of galls induced by *Subanguina picridis*. Data represent the average number of galls per dish 26 days after inoculation.

creased. As such, the resistance of the host to nematode infection increases as the plant ages. Watson (14) suggested that rapidly elongating shoots of perennial plant species are probably immune to attack from *S. picridis*. Increased cell division, coupled with an increased rate of differentiation during the elongation of perennial shoots, probably occurred too rapidly for the nematode to alter the normal plant development.

Slow-growing plants are required for *S. picridis* (14). Because Russian knapweed plants receiving a low level of nitrogen fertilization did not exhibit vigorous growth of the primary shoot, galls formed on these plants but did not form on plants receiving a high nitrogen level (14). In the present study, culturing *S. picridis* on MSG and B5G media resulted in faster and stronger shoot growth in the MSG medium than in B5G medium, but gall formation rate was lower and the gall size was smaller in MSG medium than in B5G medium. The significant feature of MSG medium is its very high content of nitrate, ammonium nitrogen, and potassium. The general concentrations of inorganic nutrients in B5G medium are lower. Young shoots under slow growth are necessary for this nematode to induce galls.

This study demonstrates that temperature and host growth pattern influence the rate at which *S. picridis* induces galls on Russian knapweed. These results suggest ways to optimize mass rearing of this nematode, information that is important in developing *S. picridis* as a successful biological control agent of Russian knapweed.

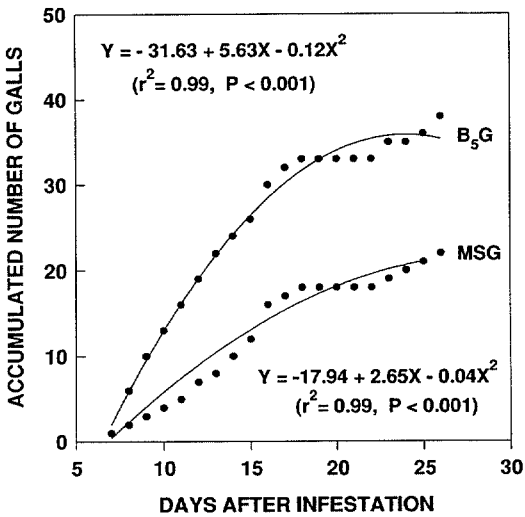

FIG. 5. The effect of MSG and B5G media on *Subanguina picridis* gall induction on Russian knapweed. Gall numbers were accumulated from 10 replicates with a total of 30 shoots.

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