Comparison of the saponin contents between various ginseng cultured tissues and original plant

Callus or tissue	Growth ratio*	Dry wt (g) per 100 g fr. wt	Saponin content (mg) per 100 g fr. wt Rb group	Rg group**	Total	Rb group / Rg group	Total saponin per dry mass (wt %)
Static							
DK callus (Fig. 1a)	4.30	2.48	2.4	8.4	10.8	0.29	0.44
K 1 shoot (Fig. 1d, e)	5.81	2.96	15.1	22.3	37.4	0.67	1.26
IBA 1 root (Fig. 1f)	3.40	3.09	27.6	25.1	52.7	1.10	1.71
Suspension							
IBA 2 K 0.1 root (Fig. 1g)	6.22	6.31	41.6	38.8	80.4	1.07	1.27
Plant							
Aerial part (stem and leaf)		9.53	21.7	62.1	83.8	0.35	0.88
Root		23.91	59.0	37.3	96.3	1.58	0.40

* Growth ratio was determined by the increase of the fresh weight after 4-week culture. The values are the quotient of the fresh weight after 4 weeks culture and the fresh weight of the inoculum. ** The amount of Rb group was calculated as the total of ginsenosides Ra, Rb_1, Rb_2, Rc and Rd, having protopanaxadiol as the sapogenin, and Rg group was calculated as the total of ginsenosides Re, Rf, Rg_1, Rg_2 and Rh, having protopanaxatriol. Each value in the cultured tissues shows the average of duplicate estimations in 4 flasks of 3 different cultures. Each value for the original plant is the average of duplicate estimations in 3 different samples.

tion, 2500–4000 lx, 16 h/day with warm fluorescent light in a phytotron cabinet, the compact calli actively generated many green shoots. Figure d shows differentiation of shoots progressing stepwise from left to right. The shoot formation was actively promoted under the same culture conditions as those described above, and finally many clonal plantlets were formed in a flask (fig. e). On the other hand, when the compact calli were transferred to the medium supplemented with IBA (indole-3-butyric acid) 1 ppm (IBA 1 medium) and cultured for several generations on the same medium in the dark, many roots were formed as shown in figure f. Subsequently, the roots were separated and subcultured on the same medium, and finally cultured in the liquid medium (fig. g). It was observed under a microscope that the morphological structures of the isolated roots (named IBA 1 strain) closely resembled those of the original plant. Thereafter, the roots were cultured on media containing various auxins and cytokinins or their combinations as described in our earlier paper[5]. The most efficient saponin production by the ginseng cultured tissues was observed in the combination of IBA 2 ppm and kinetin 0.1 ppm (named IBA 2 K 0.1 medium). The roots cultured under the best conditions finally grew to 9–12 mm in a diameter in a flask on a rotary shaker or in a vessel in a jar fermentor.

Subsequently, the saponin contents in various calli and differentiated tissues were determined according to the TLC method described in our earlier papers[3–5]. The values are shown in the table in comparison to the saponin content of the native plant. As a result, it was demonstrated that the shoots (K 1) and the roots (IBA 1) produce larger amounts of saponins than the original callus (DK callus); 3.5 times as much in K 1 tissues and 4.9 times in IBA 1. The saponin content in suspension culture (IBA 2 K 0.1 medium) was comparable to those of the aerial part and the root of the plant on a fresh weight basis. On the other hand, the saponin content of the cultured roots on a dry weight basis was 1.71% in static culture and 1.27% in suspension, and those were 3–4 times higher than that in the plant root, 0.40%. Moreover, the ratio of the ginsenoside Rb group, which has protopanaxadiol as the sapogenin, to the Rg group, protopanaxatriol, was calculated for a quality evaluation of the ginseng saponin, and compared between the various cultured tissues and the native material. The ratios in DK callus and K 1 shoot resembled those in the aerial part of the native plant, while in the root cultures, i.e. IBA 1 root and IBA 2 K 0.1 suspension, they resembled that in the plant root.

Two other groups[7,8] have already reported that shoots and roots are differentiated from ginseng callus cultures. However, all the tissues developed spontaneously from the primary callus immediately after the callus induction. This experiment sheds light for the first time on the systematic methods suitable for obtaining plantlets, or shoots and roots, which have the desired quality, from the ginseng callus subcultured for several generations, over 5 years, after the induction. Now, we are trying to grow plants on soil from the differentiated plantlets.

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Cattle grazing behavior regulates tick populations

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Summary. Analysis of field population data showed that host-seeking larvae of the tick *Boophilus microplus* were less successful in attaching to their hosts when larval densities were high. Experimental results showed that cattle hosts detected and avoided high densities of larvae in pasture. The finding reveals a previously unknown population-regulating mechanism for ticks, which are important pests of livestock.
Key words. Parasitism; behavior; population; tick; cattle; host; *Boophilus;* density dependence.

Populations of animals are stabilized by declining fecundity and reduced survival with increasing population density; a phenomenon known as density-dependent regulation. In the case of the cattle tick, *Boophilus microplus,* survival is known to be reduced with increasing densities by the host's immune response[1] and at extreme densities by the deaths of excessively infested hosts[1,2]. We now present field evidence of a further mechanism: avoidance by the host of heavy concentrations of tick larvae on pasture. We then demonstrate experimentally that cattle do avoid such concentrations.

B. microplus has a simple life-cycle. After feeding on the blood of cattle, the replete female tick drops from the host and lays about 2000 eggs at the base of a pasture plant. The larvae ascend the pasture, without dispersing laterally, to await a passing host. They transfer to animals which brush past them and a proportion succeed in feeding and mating to complete the life-cycle.

The tick, which is an important pest of cattle throughout the tropics, has been the subject of a long-term population study and the information is being incorporated into mathematical models[3]. In order to test whether the known processes could account for observed variation in the sizes of natural populations, field data[4] on *B. microplus* were collected over periods of up to 3 years from 6 herds of cattle. The cattle, which had different levels of tick resistance associated with varying proportions of *Bos indicus* (zebu) and *Bos taurus* (European) genes, grazed in separate paddocks with 3 herds at each of 2 locations in Queensland, Australia. The numbers of mature female ticks dropped into each paddock by each herd were estimated by weekly counts of those ticks on the cattle which were sufficiently mature to complete engorgement and drop from the host in the next 24 h. In addition, the numbers of larvae produced per female tick in the pastures were estimated from larvae recovered from mature ticks placed in the pasture adjacent to the paddocks. These measurements enabled us to calculate the numbers of larvae available to attach to the cattle in each paddock. The survival of larvae on the host was measured[1] every 2 months by removing the cattle from the paddocks for 2 weeks, infesting each animal with 20,000 larvae and counting the numbers of female ticks which matured. The numbers of larvae picked up from the pasture could then be estimated by working back from the observed numbers of ticks maturing from the natural infestation. The results (fig. 1) indicated that a much smaller proportion of the available larvae were picked up by the cattle at higher densities, whereas one would expect a fixed proportion if density was not important. Each point in figure 1 is the mean for 8 weeks. Fewer points were available for the *B. taurus* herds, as populations of larval ticks built up so rapidly in their paddocks that they threatened the survival of the cattle. The cattle were sprayed with a pesticide and the observations on those herds were discontinued. One possible explanation for the smaller proportion of larvae picked up at high densities was that the cattle were avoiding high concentrations of larvae in the pastures. The hypothesis was supported by an observation in the literature that cattle refused to graze in a small paddock which had been seeded with large clumps of tick larvae[5,6].

To measure the effect, an experiment was set up with 10 clumps of six numbers of larvae (0, 1000, 3000, 9000, 27000, 81000) in circles of pasture 1 m in diameter. The circles were outlined by a mown strip and arranged in a grid pattern in a 0.3 ha paddock. After release into the pasture, the larvae immediately ascended the grass over an area of about 600 cm^2 and sheltered beneath leaves. Lateral dispersal by such tick larvae is negligible[7].

Two different groups of twelve *Bos indicus* × *B. taurus* bulls with a history of tick infestation were used on each of 2 successive days. Each group was left in the paddock for 24 h. During the

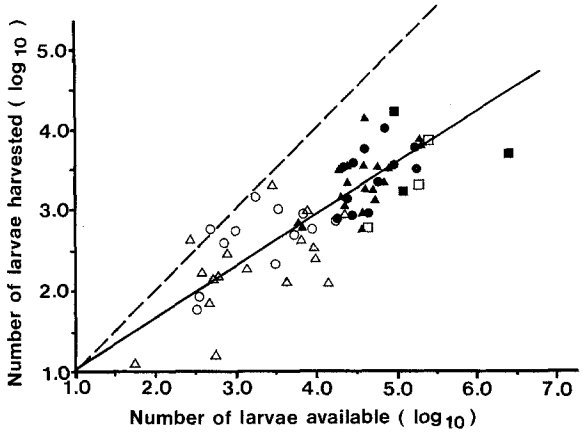

Figure 1. Relationship between larvae estimated to be available in each paddock during each 8 week period and those estimated to have been picked up by cattle. Data are given for 3 herds (□) *Bos taurus;* (○) ¾ *B. taurus* × ¼ *B. indicus;* (△) ½ *B. indicus* × ½ *B. taurus* at each of 2 locations. Solid symbols represent Central Queensland and empty symbols Southern Queensland. The relationship expected if the number of larvae picked up was proportional to the number available is shown as a broken line with slope of 1.0. The fitted solid line has a slope of 0.6.

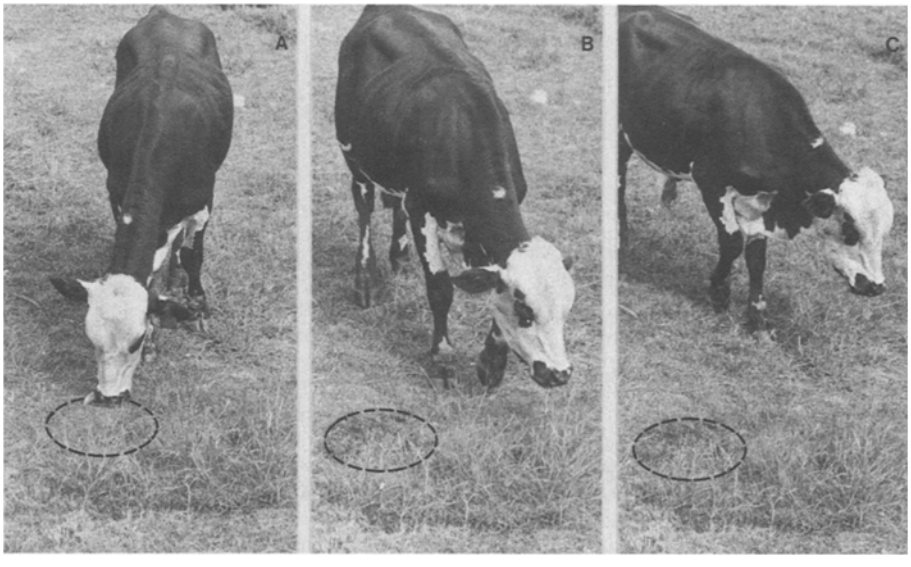

Figure 2. Avoidance by a bull of a large clump of tick larvae (circled) which were stimulated by the host's presence to emerge from shelter onto grass tips and become visible to the host (b&c). The photographs were taken in quick succession.

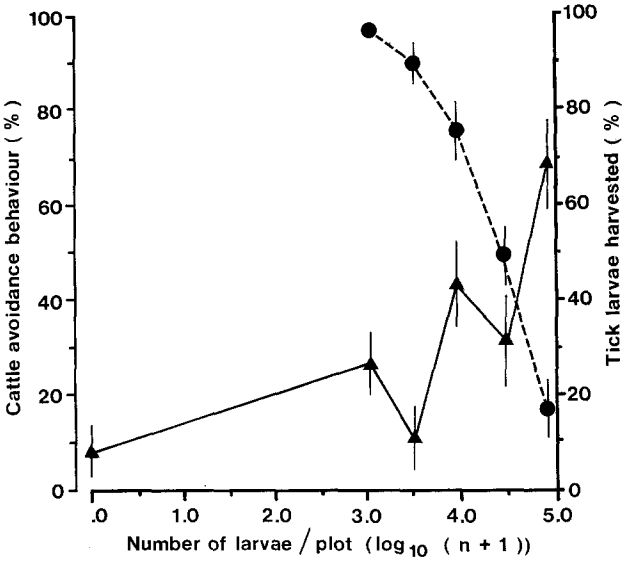

Figure 3. The effect of larval tick density on the incidence of avoidance behavior of cattle (△—△) and on the consequent percentages of larvae which were harvested (●—●). Vertical lines show ± 1 standard error.

first 3 h each day, the response of cattle encountering circles was recorded as 'obvious avoidance' when they changed direction abruptly on entering a circle, or 'no response' when they did not do so. An animal was considered to have entered a circle if its nose had entered. The observers were not told which circles contained ticks. After the 48 h of grazing, the tick larvae were recovered by clipping grass tips, using a vacuum cleaner and sweeping with flannelette bats. The procedure is known to recover about 90% of all larvae present[8]. The numbers of larvae were then estimated volumetrically in the laboratory.

Avoidance by the cattle was obvious and highly predictable when large numbers of tick larvae were present (fig. 2). Responses varied with the temperament of the individual animals and ranged from turning away or reversing to subtle bypassing movements. First awareness of the ticks was indicated by various signs of increased alertness. Vision appeared to be important in detecting the dark brown larvae, as they became clearly visible when they moved onto the upper surface of grass blades in response to the presence of a host (fig. 2).

Both the proportion of avoidance reactions of cattle and the percentage of larvae harvested by the cattle were strongly density dependent (fig. 3). Under normal grazing conditions, high concentrations of tick larvae can occur on pastures, because most mature female ticks usually drop off their host in the vicinity of overnight camp sites where cattle awaken at first light[9]. Whilst cattle appear unable to detect the larval progeny of a single female of *B. microplus* (2000 larvae), they are capable of detecting heavily contaminated foci of tick larvae. Similar avoidance of dense concentrations of ticks by nesting birds[10] and of mosquitoes by intolerant hosts of various species[11] has been reported previously. However our observations differ in that the cattle avoided the ticks before they came into physical contact with them.

We conclude that our results reveal a third regulating mechanism for tick populations. The three mechanisms in sequence are 1) avoidance of larvae on pastures by the host, 2) reduced survival and potential fecundity of ticks on the host and, as a last resort, 3) mortality of the most susceptible hosts. The current findings enable refinement of tick population models[3] as well as highlighting a population regulating mechanism that may have wider relevance in parasitology.

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Anthraquinone pigments from a conidiating mutant of *Trichoderma viride**

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Summary. Two pigments responsible for the yellow-orange color of a brown conidiating mutant of the deuteromycete *Trichoderma viride* were isolated and spectroscopically identified as 1,3,6,8-tetrahydroxyanthraquinone (**I**) and 1-acetyl-2,4,5,7-tetrahydroxy-9,10-anthracenedione (**II**). Both compounds are known substances but were not yet reported as metabolites of this fungal species. Their relationship to other anthraquinones produced by *T. viride* is discussed.

Key words. Trichoderma viride; conidiating mutant; anthraquinones.

The wild type of the deuteromycete *Trichoderma viride* produces dark-green conidia. Their formation is induced by light pulses[1]. Using UV radiation, a series of mutants were prepared, belonging either to the non-conidiating or to the conidiating types. The conidia of the latter type mutants are white, yelow or brown[2–4]. One mutant of the last type was found to accumulate indicator-like pigments both during and after conidiation.

A stationary culture of *T. viride* mutant CCM F-742 was grown