JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Monomers of Condensed Tannins Affect the Larval Exsheathment of Parasitic Nematodes of Ruminants

SEVERINE BRUNET AND HERVE HOSTE*

Unité Mixte de Recherches 1225 INRA/DGER, "Interactions Hôte Agents Pathogènes", École Nationale Vétérinaire de Toulouse, 23 chemin des Capelles, F31076 Toulouse, France

The current control of parasitic nematodes in small ruminants relies on the use of chemical anthelmintics, but the development of resistance requires research in alternatives. Bioactive plants represent one of those solutions. Previous results suggest that plants rich in condensed tannins (CTs) might have direct anthelmintic effects. However, the relationships between the biochemistry of active compounds and the mechanisms of action remain to be elucidated. Therefore, this study examined in vitro the effects of different CT monomers on the exsheathment of infective larvae for two nematode species. Monomers of prodelphinidins were more potent inhibitors of exsheathment than those of procyanidins. More severe inhibition was also found with galloyl derivatives. These results suggest that the number of free hydroxy groups of CT monomers is a key factor in interactions with parasite larvae. Comparison of effects between the two nematodes suggests different susceptibility to monomers depending on species, which might be related to the protein composition of sheaths.

KEYWORDS: Nutraceutical; parasitic nematode; flavan-3-ols; condensed tannins; larval exsheathment; *Haemonchus contortus*; *Trichostrongylus colubriformis*; prodelphinidins; procyanidins

INTRODUCTION

Nematodes of the digestive tract represent a major threat in the breeding of grazing ruminants. Up to now, the control of these parasites was generally based on the repeated use of chemical anthelmintics. However, due to the constant development of anthelmintic resistance in worm populations in small ruminants (*1*, *2*), the search for alternative solutions to chemical treatments is nowadays a necessity to achieve a more sustainable control of this parasitism (*3*). In the past decade, evidence has accumulated indicating that some bioactive plants might possess anthelmintic properties and, thus, represent a promising alternative to chemotherapy when used as nutraceuticals (*4*−*7*). Among the different secondary metabolites present in bioactive plants, the potential role of tannins in the observed effects has usually been emphasized.

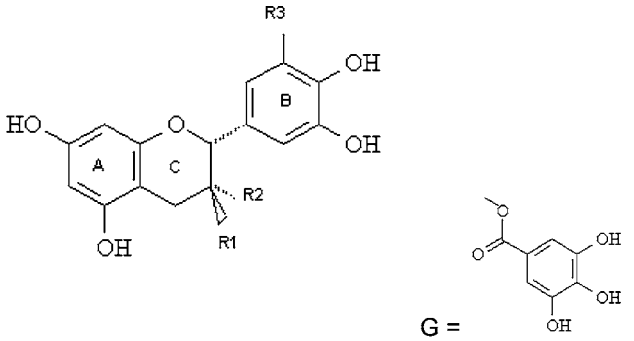
Previous results on the activity of bioactive plants against parasitic nematodes have been acquired from both in vivo and in vitro studies. Most in vivo results have been acquired in ruminants infected with adult worm populations (*7*, *8*). They have shown that the consumption of tannin-rich plants by ruminants was generally associated with a significant decrease in nematode egg output and, to a lesser extent, in female worm fecundity (*8*−*11*). In contrast, only a few in vivo studies have examined in controlled conditions the consequences of a tannin-rich digestive environment on the nematode third-stage infective larvae (L3). In goats, when quebracho was given prior to and/or during infection with L3, significant reductions in the larval establishment were observed (*10*).

Only a few in vitro results have confirmed the effect of tannin-rich plant extracts on adult nematodes (*12*). In contrast, many in vitro results performed on the L3 have shown that their motility was significantly and adversely modified after contact with extracts of tannin-rich legume forages (*13*, *14*), woody plants (*12*), or extracted condensed tannins (*15*−*18*). In most studies, these negative effects on the larvae disappeared after the addition of inhibitors of tannins, such as polyethylene glycol (PEG), to the extracts (*12*, *14*). These latter results have thus substantiated the hypothesis that, among the different plant secondary metabolites, the condensed tannins (CTs) might play a central role in the anthelmintic activity. The fact that many plant extracts showed an activity against the worms in in vitro conditions supports the hypothesis of direct anthelmintic effects. However, the mechanisms of action of CTs on the parasitic nematodes remain obscure in regard to interactions with the physiology of the different parasitic stages.

For trichostrongyle nematodes, the L3 exsheathment is a key process in the life cycle because it is the step making the transition from the free-living to the parasitic stages (*19*, *20*). Studies on the kinetics of larval exsheathment have emphasized that the phenomenon has to occur within a restricted time frame and that any disturbing factors might reduce the parasite establishment in the host (*21*, *22*). This is why it is important to improve our understanding of the mechanisms underlying the interactions between tannins and the larval exsheathment. A better characterization of the biochemical compounds involved

* Corresponding author [e-mail h.hoste@envt.fr; telephone +(33) 05-61-19-38-75; fax +(33) 05-61-19-32-43].

10.1021/jf0610007 CCC: $33.50
Published on Web 09/02/2006



	R1	R2	R3	Number of OH groups	FW
catechin	OH	H	H	5	290,3
epicatechin	H	OH	H	5	290,3
gallocatechin	OH	H	OH	6	306,3
epigallocatechin	H	OH	OH	6	306,3
catechin gallate	G	H	H	8	442,4
epicatechin gallate	H	G	H	8	442,4
gallocatechin gallate	G	H	OH	9	458,4
epigallocatechin gallate	H	G	OH	9	458,4

Figure 1. Chemical structure of monomers of condensed tannins: flavan-3-ols and flavan-3-ol gallates.

is also required for a pertinent use of those nutraceuticals in farm conditions.

In a recent study, we examined the possible interactions of extracts from four tannin-rich plants with the L3 exsheathment. Results indicated that the contact with most plant extracts was associated with either a partial or a total inhibition of the larval exsheathment, when artificially provoked in in vitro conditions. This was described for larvae of both the abomasal species, *Haemonchus contortus*, and of the intestinal species, *Trichostrongylus colubriformis* (*23*). The addition of PEG to extracts usually led to a restoration toward the rate of exsheathment similar to control values. These results thus suggest that (i) extracts of tannin-rich plants might interfere with the very early step of the host invasion, (ii) the effects were nonspecific of parasite species, and (iii) tannins were largely involved in this inhibitory process. However, the precise nature of the tannins involved remains unknown.

CTs are polymers of flavan-3-ol units with a wide range of structural variations depending on the monomeric units and the degree of polymerization (*24*−*26*). The biochemical structure of flavan-3-ols can differ by the number of phenolic groups on the B-ring, the 2,3 stereochemistry of the C-ring, and the presence of a galloyl group attached to the C ring (see **Figure 1**) (*27*). The monomers of procyanidins (PC) are either catechin or epicatechin and their galloyl derivatives, whereas the constituents of prodelphinidins (PD) are gallocatechin, epigallocatechin, and their galloyl derivatives. Overall, tannins are defined as polyphenols, which have the property to bind with proteins or polysaccharides. Differences in the biochemical structure, related to the nature of monomers, have been shown to modulate the biological properties of CTs, in particular, their ability to bind to proteins (*24*−*26*). With regard to activity on nematodes, the results of two studies also suggested that prodelphinidins were more potent inhibitors of the motility of larvae than procyanidins (*16*, *17*). However, similar information in relation to the larval exsheathment process is lacking.

The objectives of the current study were therefore (i) to compare the inhibitory effects of different flavan-3-ols and the galloyl derivatives of procyanidins and prodelphinidins on the exsheathment process of the infective L3 and (ii) to evaluate the specificity of the effects by comparing data acquired on two parasitic models, *H. contortus* and *T. colubriformis*.

MATERIALS AND METHODS

Chemicals. Monomers of procyanidins [(+)-catechin (C), (−)-epicatechin (EC), (−)-catechin gallate (Cg), and (−)-epicatechin gallate (ECg)] and prodelphinidins [(−)-gallocatechin (GC), (−)-epigallocatechin (EGC), (−)-gallocatechin gallate (GCg), and (−)-epigallocatechin gallate (EGCg)] were obtained from Sigma (St. Louis, MO).

Biological Material. The third-stage larvae were obtained, respectively, from donor goats infected with pure strains of either *H. contortus* or *T. colubriformis*. The same batch of 2−3-month-old larvae was used in the assays.

Larval Exsheathment Assay. The larval exsheathment assay was performed, as described by Bahuaud et al. (*23*), to compare the inhibitory effects of the four flavan-3-ols and the four galloyl derivatives on the exsheathment of *H. contortus* and *T. colubriformis* L3.

One thousand ensheathed nematode L3 were first incubated for 3 h at 20 °C with one of the eight molecules at the concentration of 150 μg/mL in phosphate buffer solution (PBS; 0.1 M phosphate, 0.05 M NaCl, pH 7,2). The use of PBS aimed at avoiding interference with any nonspecific effect due to pH change. After incubation, the larvae were washed and centrifuged three times in PBS, pH 7.2. Then, the larvae were submitted to the artificial process of exsheathment by contact with a solution of sodium hypochlorite (2% w/v) and sodium chloride (16.5% w/v) diluted in 1 to 300 in PBS, pH 7.2.

The kinetics of exsheathment according to the different experimental treatments was measured under microscopic observation at a magnification of ×200 by identification of the proportion of exsheathed larvae. Regular examination was performed at 10, 20, 30, 40, 50, and 60 min for *H. contortus* and at 10, 20, 30, 40, 50, 60, and 70 min for *T. colubriformis* after contact with the solution for exsheathment. For each monomer, six replicates were run per assay to examine the changes in proportion of exsheathed larvae with time. In addition to the eight molecules tested, negative controls (L3 in PBS) were run in parallel.

Statistical Analyses of the Results. The statistical comparisons of differences in mean of the exsheathment rates were based on the results from the six replicates, depending on larval treatments and time. The statistical differences across time were assessed through the general linear model (GLM) procedure using Systat 9 software (SPSS Ltd.).

In addition, a multivariate analysis [multiple correspondence analysis (MCA)] was performed using the Systat 9 software (SPSS Ltd.) to obtain a synthetic description of the relationships between the effects on larval exsheathment in the two nematode species and the main characteristics of the monomer structures.

The six variables composing the column of the matrix used for the MCA were categorical. They included the nematode species (*H. contortus* or *T. colubriformis*), the total number of free hydroxy groups (Tot5, Tot6, Tot8, or Tot9), the possible structure variations in either R1, R2, or R3 groups (H, OH, or G), and the observed effects measured on the larval exsheathment (inhibition, delay, or nonsignificant effect). The 16 rows (individual data) of the matrix corresponded to the 8 different monomers assayed on the two nematode species.

RESULTS

Effects of Various Flavan-3-ols and Their Galloyl Derivatives on the Nematode Species. In controls, >90% of the *H. contortus* larvae were generally exsheathed after 60 min of contact with the solution for exsheathment (see **Figures 2** and **3**). For *T. colubriformis*, a similar pattern was observed but slightly delayed because <80% of the larvae were exsheathed at 60 min, whereas the proportion was >90% after 70 min (see **Figures 2** and **3**).

Catechin had no effect on the larval exsheathment for both nematode species (**Figure 2A**,**E**). Catechin gallate had no effect on *H. contortus* larvae (**Figure 3A**), but provoked a significant delay ($P < 0.01$) in the exsheathment rate of *T. colubriformis* (**Figure 3E**).

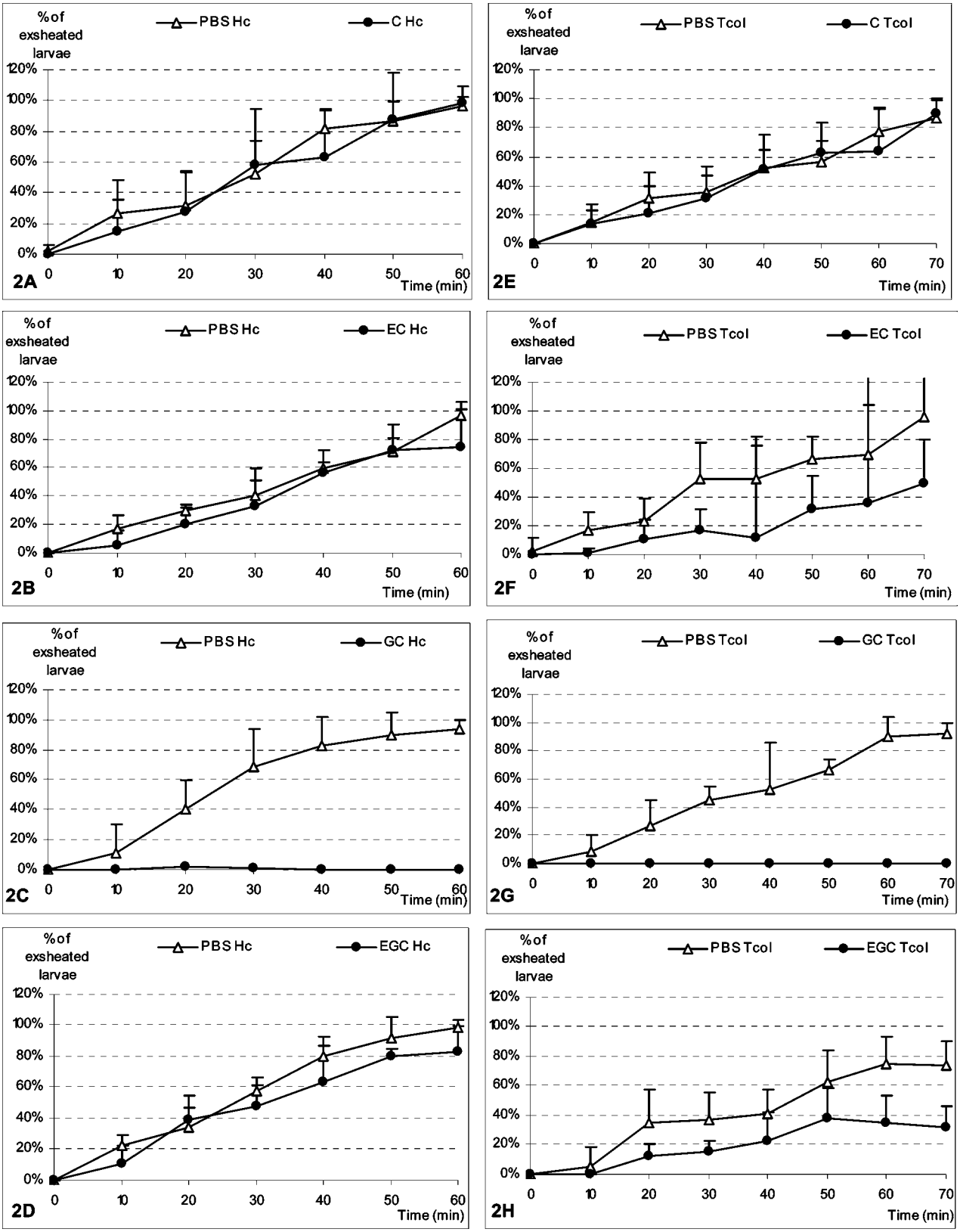


Figure 2. Effects of 3 h of contact of *H. contortus* third-stage larvae (**A**–**D**) and *T. colubriformis* third-stage larvae (**E**–**H**) with flavan-3-ols on the process of artificial in vitro exsheathment.

In the case of epicatechin, the larval exsheathment was not affected for *H. contortus* larvae (**Figure 2B**) but was significantly delayed ($P < 0.01$) for *T. colubriformis* larvae (**Figure 2F**). In contrast, the epicatechin gallate induced a total, significant inhibition ($P < 0.001$) of the process for both *H. contortus* (**Figure 3B**) and *T. colubriformis* (**Figure 3F**).

The 3 h of contact with gallocatechin and with its galloyl derivative led to a total inhibition ($P < 0.001$) of the exsheathment for both nematode species, because no larvae were exsheathed after 60 min for *H. contortus* (**Figures 2C** and **3C**) and after 70 min for *T. colubriformis* (**Figures 2G** and **3G**). In contrast, for the controls, the mean percentages ranged, respec-

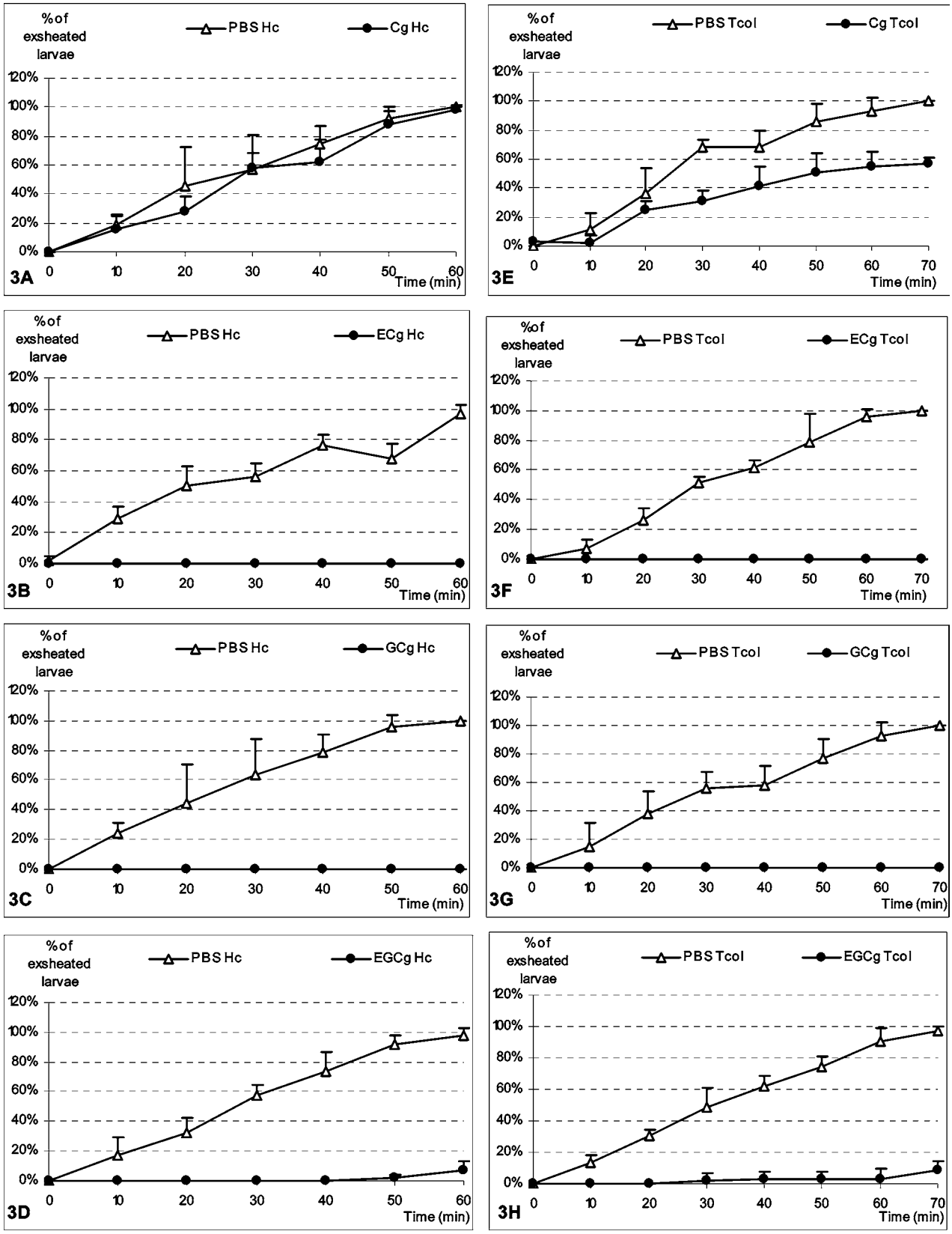


Figure 3. Effects of 3 h of contact of *H. contortus* third-stage larvae (**A**–**D**) and *T. colubriformis* third-stage larvae (**E**–**H**) with flavan-3-ol gallates on the process of artificial in vitro exsheathment.

tively, from 94 to 100% for *H. contortus* and from 92 to 100% for *T. colubriformis*.

For *H. contortus* (**Figure 2D**), the epigallocatechin treatment had no effect on the exsheathment rate, whereas the phenomenon was significantly ($P < 0.01$) delayed for *T. colubriformis* (**Figure 2H**). With the galloyl derivative, a nearly total inhibition ($P < 0.001$) of the process was observed for both parasite species (**Figure 3D**,**H**) because only 6% of the larvae were exsheathed after 60 min for *H. contortus* and only 8% were exsheathed after 70 min for *T. colubriformis*.

In addition, a different trend in the effects on the larval exsheathment was observed for monomers of both prodelphini-

Table 1. Statistical Results on the Larval Exsheathment after 3 h of Incubation of *H. contortus* and *T. colubriformis* Third-Stage Larvae with Catechin (C), Epicatechin (EC), Gallocatechin (GC), Epigallocatechin (EGC), or Their Galloyl Derivatives (g)

	procyanidins				prodelphinidins			
	C	EC	Cg	ECg	GC	EGC	GCg	EGCg
H. contortus	NS	NS	NS	$P < 0.001$	$P < 0.001$	NS	$P < 0.001$	$P < 0.001$
T. colubriformis	NS	$P < 0.01$	$P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

dins and procyanidins, depending on the parasite species, because significant effects on the exsheathment process were more frequent for *T. colubriformis* larvae than for *H. contortus*. EC, Cg, and EGC did not affect the abomasal larvae, whereas these monomers induced a significant delay in the rate of *T. colubriformis* exsheathment.

To summarize, the effects on the larval exsheathment were overall more pronounced and/or more frequently significant with the monomers of prodelphinidins than with monomers of procyanidins, with the flavan-3-ol gallates compared to the flavan-3-ols (**Table 1**), and, last, on the intestinal larvae compared to the abomasal ones.

Multiple Correspondence Analysis. In MCA, a plane describing the relationships between the variable is composed of two axes. Each axis corresponds to a linear combination of variables of the matrix. The main variables contributing to create axis 1 were "the effect", "the total number of OH group", and "the structure of the R3 group". In contrast, the two major variables contributing to axis 2 were "the structure of R1" and "the structure of R2".

The observed variables were projected on the plane defined by the combination of axes 1 and 2 (**Figure 4**), which represented 43.31% of the whole variance. The main objective was to analyze the overall relationships between the different categories of effects on exsheathment with other variables (**Figure 4**). Variables that are positively related are located in the same area of the plane.

The total inhibition (Inh.) of the larval exsheathment was in proximity with the presence of the hydroxy group in R3 (R3OH), the presence of nine OH groups (Tot9) and, to a lesser extent, the presence of a galloyl group (R2G and R1G) in the molecule.

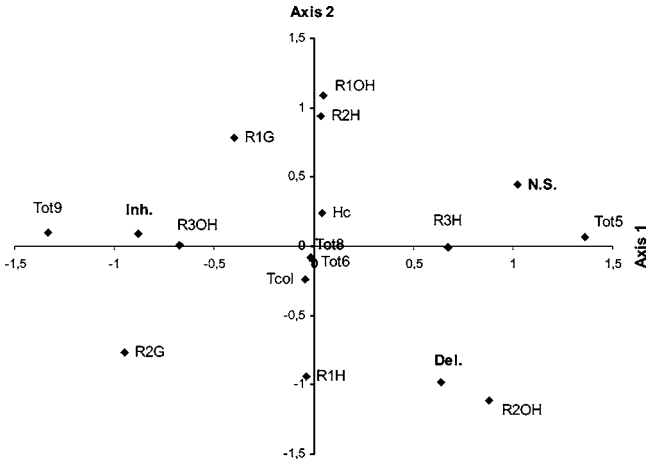


Figure 4. Principal plane of interactions obtained from the MCA applied on a matrix composed of six variables describing the structure of flavan-3-ol units and the effects on the exsheathment of either *H. contortus* or *T. colubriformis* third-stage larvae. Abbreviations: effects on the larval exsheathment (Inh., Del., and N.S. designed, respectively, a total inhibition, a delay, or no significant effect); nematode species (Hc, *H. contortus*; Tcol, *T. colubriformis*); total number of OH groups in the biochemical structure (Tot5, Tot6, Tot8, or Tot9); structural variations of R1 group (H, OH, or galloyl group) (R1H, R1OH, or R1G); structural variations of R2 group (H, OH, or galloyl group) (R2H, R2OH, or R2G); structural variations of R3 group (H or OH) (R3H or R3OH).

The delay in exsheathment (Del.) was associated with the presence of the OH group in R2.

Finally, the absence of effect (N.S.) was related with a low number of total OH groups (Tot5) and with the presence of hydrogen in R3 (R3H).

DISCUSSION

One of the first pieces of information gained from this study was that monomers of CTs interact with the exsheathment of the nematode third-stage larvae, as was previously shown with whole extracts of four tannin-rich plants (*23*). In both studies, due to the affinity of tannins (*24*−*27*) and/or their monomers for proteins (*28*), it can be assumed that the changes in the exsheathment rate resulted from interactions with proteins of nematode larvae. Results of the current study provide additional information on the influence of the biochemical structure of flavan-3-ols on these interactions.

In a recent study, Molan et al. (*16*) have emphasized that differences in the structure of flavan-3-ols and flavan-3-ol gallates modulated the inhibitory effects on the larval development of *T. colubriformis* and/or the motility of the larvae. From these in vitro results, it has been proposed that the variations in in vivo effects on nematode populations observed with various tannin-rich plants could be related to differences in the biochemical structure of CTs. Overall, the bioactive plants with the highest activity against gastrointestinal nematodes in ruminants were those containing a high prodelphinidin/procyanidin ratio (*5*, *7*, *16*, *29*).

The first aim of our study was therefore to explore the hypothesis that the biochemistry of CT monomers might also modulate the effects on larval exsheathment. Our results suggest that indeed a relationship exists between the structure of monomers and the consequences on the artificially induced exsheathment. This has been substantiated by comparison of results obtained both with monomeric units of prodelphinidins (PD) versus procyanidins (PC) or between flavan-3-ols and the galloyl derivatives.

First, the comparison of the effects due to monomers of PDs and PCs indicates that the trihydroxyflavan-3-ols (PD units) were usually more active than the dihydroxyflavan-3-ols (PC units). For example, for the same 2,3-trans stereochemistry, gallocatechin (PD unit) induced a total inhibition of exsheathment for both nematode species, whereas catechin (PC unit) did not provoke any change compared to controls. The same situation was observed for gallocatechin gallate (PD unit) versus catechin gallate (PC unit) for *Haemonchus* larvae. For *T. colubriformis*, the GCg (PD unit) also provoked a total blockade of exsheathment, whereas the phenomenon was only delayed with the Cg (PC unit). The trend was less clear when the effects due to epigallocatechin (PD unit) and epicatechin (PC unit) or their galloyl derivates were compared. For the two nematode species, similar effects were found with the two monomers. On the other hand, the two galloyl derivatives induced a total inhi-

bition of exsheathment, whatever the parasite species, making comparisons difficult to interpret.

Moreover, for both larval species, the comparison of consequences on exsheathment provoked by EC gallate and EGC gallate versus EC and EGC confirmed that galloylation of flavan-3-ols was often associated with higher activity against the nematodes as previously suggested (*16*, *17*). This was also illustrated by comparing the effects of catechin gallate and catechin on *T. colubriformis*. For the last possible comparison (GCg versus GC), the differences were absent because a total inhibition was observed on both parasite species.

In contrast, the stereochemistry of the C-ring conformation did not seem to be a significant factor modulating the reactivity of CT units with the larvae, as previously evoked by Molan et al. (*16*). In the case of PD units, the 2,3-trans stereochemistry (GC) appeared to be more effective than the 2,3-cis stereochemistry (EGC), for both nematode species. However, this trend was not observed with the PC units or with the gallates.

The second objective of our study was to examine whether differences in activity of the CT monomers on the exsheathment process exist depending on the parasite species. From the results obtained with the eight molecules on the abomasal and the intestinal species, three molecules (Cg, EC, and EGC) provoked a significant delay on the exsheathment of *T. colubriformis* larvae, but had no effect on *H. contortus*. This result suggests possible differences in susceptibility according to the worm species and tends to confirm previous observations. First, several in vivo studies examining the consequences on nematodes of the distribution of tannin-rich resources to infected animals have shown differences in effects depending on the parasite species and its anatomical location. For example, in sheep (*8*) or in goats receiving quebracho (*10*, *11*), significant effects on the worm populations of the small intestine (*T. colubriformis* and *Nematodirus battus*) have been described, whereas no effect was found on the abomasal species, *H. contortus*. Similarly, results of several studies, which compared the in vitro anthelmintic effects of tannin-rich extracts on different nematode L3, based on the larval migration inhibition assay have shown differences in susceptibility according to the parasite species. This was observed with whole plant extracts from forages (*13*), with woody plants (*12*), or with tannins extracted from plants (*17*).

It is known that the larval exsheathment process results from the action of some enzymes, including proteinases, on the proteins of the larval sheath (*30*, *31*). Information on the biochemical composition of the nematode larval sheath has emphasized that collagen-like proteins and non-collagen-like proteins (cuticlin), both possessing high contents of proline and hydroxyproline, were two major components (*32*, *33*). On the other hand, variations in the affinity of different flavan-3-ols units have recently been underscored using poly(L-proline) as model (*28*). At a molecular level, it can thus be hypothesized that differences in susceptibility to CT monomers between *H. contortus* and *T. colubriformis* might result from specific differences in composition of the sheath and/or exsheathment enzymes.

As previously emphasized, whatever the nematode, more marked effects on larval exsheathment have generally been associated with the prodelphinidin monomers and than with the galloyl derivatives. This was also illustrated by the MCA (**Figure 4**), which showed that the total inhibition of exsheathment was associated with the total number of hydroxy groups, and hence the molecular weight of the molecules, as well as with the presence of one hydroxy group in R3, which characterizes the PD units, and to a lesser extent with galloylation of the monomers. The presence of a high number of OH groups in the flavan-3-ol structure might favor hydrogen bonds with the proteins of larvae. Moreover, the presence of gallate has also been proposed to be involved in hydrophobic interactions (*25*, *28*). This is providing information to orient the choice of plant resources to be exploited as potential nutraceuticals against these major parasites of domestic ruminants.

To improve our understanding of interactions between CTs and the parasites, further works on the mechanism of action are required. Although acquired on CT monomers, and not directly on CTs, the current results provide some indications on factors modulating these interactions. Care has been taken to apply concentrations that were similar or close to the values applied in previous in vitro assays (*16*), in order to correspond to in vivo values reported in the gut digesta (*16*, *17*). However, the current in vitro results need to be verified and completed through in vivo studies, in particular by using cannulated animals.

ABBREVIATIONS USED

C, (+)-catechin; Cg, (−)-catechin gallate; CTs, condensed tannins; EC, (−)-epicatechin; ECg, (−)-epicatechin gallate; EGC, (−)-epigallocatechin; EGCg, (−)-epigallocatechin gallate; GC, (−)-gallocatechin; GCg, (−)-gallocatechin gallate; L3, third-stage infective larvae; PBS, phosphate buffer solution; PC, procyanidin; PD, prodelphinidin.

ACKNOWLEDGMENT

We sincerely thank Dr. I. Mueller Harvey from the University of Reading for her involvement and pertinent advice to improve the manuscript.

LITERATURE CITED

(1) Jackson, F.; Coop, R. L. The development of anthelmintic resistance in sheep nematodes. *Parasitology* **2000**, *120*, 95−97.
(2) Kaplan, R. M. Drug resistance in nematodes of veterinary importance: a status report. *Trends Parasitol.* **2004**, *20*, 477−481.
(3) Waller, P. J. Sustainable nematode parasite control strategies for ruminant livestock by grazing management and biological control. *Anim. Feed Sci. Technol.* **2006**, *126*, 277−289.
(4) Kahn, L. P.; Diaz-Hernandez, A. Tannins with anthelmintic properties. Tannins in livestock and human nutrition. *ACIAR Proceeding 92*; International workshop: Adelaide, Australia, 2000; pp 140−149.
(5) Min, B. R.; Hart, S. P. Tannins for suppression of internal parasites. *J. Anim. Sci.* **2003**, *81*, 102−109.
(6) Waller, P. J.; Thamsborg, S. M. Nematode control in "green" ruminant production systems. *Trends Parasitol.* **2004**, *20*, 493−497.
(7) Hoste, H.; Jackson, F.; Athanasiadou, S.; Thamsborg, S.M.; Hoskin, S. O. The effect of tannin-rich plants on parasitic nematodes in ruminants. *Trends Parasitol.* **2006**, *22*, 253−261.
(8) Athanasiadou, S.; Kyriazakis, I.; Jackson, F.; Coop, R. L. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep *in vitro* and *in vivo* studies. *Vet. Parasitol.* **2001**, *99*, 205−219.
(9) Hoskin, S. O.; Wilson, P. R.; Barry, T. N.; Charleston, W. A. G.; Waghorn, G. C. Effect of forage legumes containing condensed tannins on lungworm (*Dictyocaulus* sp.) and gastrointestinal parasitism in young red deer (*Cervus elaphus*). *Res. Vet. Sci.* **2000**, *68*, 223−230.
(10) Paolini, V.; Frayssines, A.; De La Farge, F.; Dorchies, Ph.; Hoste, H. Effects of condensed tannins on established populations and on incoming larvae of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* in goats. *Vet. Res.* **2003**, *34*, 331−339.

(11) Paolini, V.; Bergaud, J. P.; Grisez, C.; Prevot, F.; Dorchies, Ph.; Hoste, H. Effects of condensed tannins on goats experimentally infected with *Haemonchus contortus. Vet. Parasitol.* **2003**, *113*, 253−261.
(12) Paolini, V.; Fouraste I.; Hoste, H. In vitro effects of three woody plant and sainfoin on third-stage larvae and adult worms of three gastrointestinal nematodes. *Parasitology* **2004**, *129*, 69−77.
(13) Molan, A. L.; Alexander, R. A.; Brookes, I. M.; McNabb, W. C. Effect of an extract from sulla (*Hedysarum coronarium*) containing condensed tannins on the migration of three sheep gastrointestinal nematodes *in vitro. Proc. N. Z. Soc. Anim. Prod.* **2000**, *60*, 21−25.
(14) Molan, A. L.; Waghorn, G. C.; Min, B. M.; McNabb, W. C. The effect of condensed tannins from seven herbages on *Trichostrongylus colubriformis* larval migration *in vitro*. *Folia Parasitol.* **2000**, *47*, 39−44.
(15) Molan, A. L.; Hoskin, O.; Barry, T.; McNabb, W. C. Effect of condensed tannins extracted from four forages on the viability of the larvae of deer lungworms and gastrointestinal nematodes. *Vet. Rec.* **2000**, *147*, 44−48.
(16) Molan, A. L.; Meagher, L. P.; Spencer, P. A.; Sivakumaran, S. Effect of flavan-3-ols on in vitro egg hatching, larval development and viability of infective larvae of *Trichostrongylus colubriformis. Int. J. Parasitol.* **2003**, *33*, 1691−1698.
(17) Molan, A. L.; Sivakumaran, S.; Spencer, P. A.; Meagher, L. P. Green tea flavan-3-ols and oligomeric proanthocyanidins inhibit the motility of infective larvae of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis in vitro. Vet. Sci.* **2004**, *77*, 239−243.
(18) Barrau, E.; Fabre, N.; Fouraste, I.; Hoste, H. Effect of bioactive compounds from sainfoin (*Onobrychis viciifolia* scop.) on the *in vitro* larval migration of *Haemonchus contortus:* role of tannins and flavonol glycosides. *Parasitology* **2005**, *131*, 531−538.
(19) Rogers, W. P.; Somerville, R. I. The infectious process and its relation to the development of early parasitic stages of nematodes. *Adv. Parasitol.* **1968**, *6*, 327−348.
(20) Hertzberg, H.; Huwyler, U.; Kohler, L.; Rehbein, S.; Wanner, M. Kinetics of exsheathment of infective ovine and bovine strongylid larvae *in vivo* and *in vitro. Parasitology* **2002**, *125*, 65−70.
(21) Dakkak, A.; Fioramonti, J.; Bueno, L. *Haemonchus contortus* third-stage larvae in sheep: kinetics of arrival into the abomasum and transformation during rumino-omasal transit. *Res. Vet. Sci.* **1981**, *31*, 384−385.
(22) DeRosa, A. A.; Chirgwin, S. R.; Fletcher, J.; Williams, J. C.; Klei, T. R. Exsheathment of *Ostertagia ostertagi* infective larvae following exposure to bovine rumen contents derived from low and high roughage diets. *Vet. Parasitol.* **2005**, *129*, 77−81.
(23) Bahuaud, D.; Martinez-Ortiz De Montellano, C.; Chauveau, S.; Prevot, F.; Torres-Acosta, F.; Fouraste, I.; Hoste, H. Effects of four tanniferous plant extracts on the *in vitro* exsheathment of third-stage larvae of parasitic nematodes. *Parasitology* **2006**, *132*, 545−554.
(24) Bravo, L. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. *Nutr. Rev.* **1998**, *56*, 317−333.
(25) Bruneton, J., Tanins. In *Pharmacognosie, Phytochimie, Plantes Médicinales*, 3rd ed.; Tec et Doc: Paris, France, 1999; pp 369−404.
(26) Waterman, P. G. The tannins—an overview. In *Tannins in Livestock and Human Nutrition*; Brooker, J. D., Ed.; Proceedings of an International Workshop, Adelaide, Australia; Australian Centre for International Agricultural Research: 1999; pp 10−13.
(27) Mueller-Harvey, I.; McAllan, A. B. Tannins: their biochemistry and nutritional properties. *Adv. Plant Cell Biochem. Biotechnol.* **1992**, *1*, 151−217.
(28) Poncet-Legrand, C.; Edelmann, A.; Putaux, J. L.; Cartalade, D.; Sarni-Manchado, P.; Vernhet, A. Poly(L-proline) interactions with flavan-3-ols units : influence of the molecular structure and the polyphenol/protein ratio. *Food Hydrocolloids* **2006**, *20*, 687−697.
(29) Molan, A.; Duncan, A.; Barry, T.; Mc Nabb, W. C. Effects of condensed tannins and crude sesquiterpene lactones extracted from chicory on the motility of larvae of deer lungworm and gastrointestinal nematodes. *Parasitol. Int.* **2003**, *52*, 209−218.
(30) Rogers, W. P. Enzymes in the exsheathing fluid of nematodes and their biological significance. *Int. J. Parasitol.* **1982**, *12*, 495−502.
(31) Gamble, H. R.; Lichtenfels, R.; Purcell, J. P. Light and scanning microscopy of the ecdysis of *Haemonchus contortus* infective larvae. *J. Parasitol.* **1989**, *75*, 303−307.
(32) Fetterer, R. H.; Rhoads, M. L. Biochemistry of the nematode cuticle: relevance to parasitic nematodes of livestock. *Vet. Parasitol.* **1993**, *46*, 103−111.
(33) Page, A. P. The nematode cuticle: synthesis, modification and mutants. In *Parasitic Nematodes: Molecular Biology, Biochemistry and Immunology*; Kennedy, M. W., Harnett, W., Eds.; CAB: London, U.K., 2001; pp 167−193.

Received for review April 10, 2006. Revised manuscript received July 27, 2006. Accepted July 27, 2006. This work received financial support from the Région Midi-Pyrénées (Contract 030121196) and the EU Project "Healthy Hay", which are sincerely acknowledged.

JF0610007