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PREFERENCE FOR ACYANOGENIC WHITE CLOVER (*Trifolium repens*) IN THE VOLE *Arvicola terrestris*: I. EXPERIMENTS WITH TWO VARIETIES

FRANCIS SAUCY,* JACQUES STUDER,[1] VERA AERNI,[2] and BEAT SCHNEITER[3]

Department of Biology, Ecology and Biology of Organisms
University of Fribourg
Pérolles, CH-1700 Fribourg, Switzerland

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Abstract—We report experimental results showing that, under both laboratory conditions as well as in outdoor enclosures, the fossorial vole *Arvicola terrestris* preferentially feeds on acyanogenic white clover (*Trifolium repens*) when offered the choice between two varieties (Ladino and Aran) differing highly in their content in cyanogenic glycosides. We also observed that the voles adapted their diet and reduced their relative consumption of the cyanogenic variety during experiments conducted for two to three weeks in outdoor enclosures as compared to shorter tests conducted for 48 hr in laboratory cages. In addition, we report a similar preference for the acyanogenic Ladino variety for the slugs *Arion ater* and *A. subfuscus*.

Key Words—Cyanogenesis, voles, *Arvicola terrestris*, white clover, *Trifolium repens*, Ladino, Aran, *Arion ater*, *Arion subfuscus*.

INTRODUCTION

Small mammals (voles, lemmings) have long been known to exhibit "population cycles," i.e., strong fluctuations in numbers recurring at three- to four-year intervals (Krebs and Myers, 1974). The origin of these fluctuations is still considered to be an enigma (Lidicker, 1988), and more than 20 explanatory hypotheses have been proposed to account for this puzzling phenomenon (Batzli, 1992). Among

*To whom correspondence should be addressed.
[1]Present address: Fonderie 8C, CH-1700 Fribourg, Switzerland.
[2]Present address: Forgerons 6, CH-1700 Fribourg, Switzerland.
[3]Present address: Seeli, CH-1715 Alterswil, Switzerland.

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these hypotheses, the roles of food quality and of plant secondary compounds often have been evoked.

Among the many classes of plant secondary compounds that can affect herbivores, cyanogenic glycosides are of particular significance because of their acute toxicity (Seigler, 1991). Cyanogenesis (the ability of plants to produce and store cyanogenic glycosides that release HCN when hydrolyzed by a specific β-glycosidase) has been reported in many plant species (Hegnauer, 1986; Seigler, 1991). In *Trifolium repens* (white clover), cyanogenic glycosides are concentrated in the leaves and do not occur in the roots and the stems. The enzyme (linamarase EC 3.2.1.21) is located in the cell walls and the substrate (linamarin and lotaustralin) in the cells (Kakes, 1985; Kakes and Eeltink, 1985).

The genetic basis of cyanogenesis in *T. repens* and *Lotus corniculatus* is well known (Hughes, 1981), and a widespread polymorphism for cyanogenesis has been reported to occur in natural populations (reviewed in Jones, 1988). In addition, a temporal and intraplant variability in the expression of cyanogenesis has also been found in *T. repens* (Ramnani and Jones, 1985; Till-Bottraud et al., 1988).

The maintenance of the polymorphism for cyanogenesis in natural populations remains poorly understood with many observations in contradiction with Daday's hypothesis (1954) that temperature is a sufficient factor to explain this phenomenon (reviewed in Jones, 1988, 1998). As an alternative hypothesis, Ellis et al. (1977) proposed that this polymorphism could be maintained by herbivorous mollusks and suggested that cyanogenic morphs were few when slugs and snails were rare and common when mollusks were abundant. The defensive function of cyanogenesis has been documented in many studies with ample evidence that many invertebrate herbivores, mostly mollusks and insects, were deterred from feeding on cyanogenic plants (Jones, 1962, 1966; Crawford-Sidebotham, 1972). However, a critical review of the evidence indicates that a preference for acyanogenic plants has been shown for only 53% of the herbivore species tested and that many studies in which such a preference has been allegedly found lack an appropriate statistical treatment (Hruska, 1988).

Apart from experiments by Jones (1962, 1966), who has tested two individual voles (*Microtus agrestis*), few studies involving mammals have been conducted. Differential grazing between cyanogenic and acyanogenic plants has been suggested from field studies in deer and rabbits (Corkill, 1952; Cooper-Driver and Swain, 1976), but no selective eating could be detected in sheep (Corkill, 1952). Sherbrooke (1976) reports field and laboratory evidence for differential consumption of cyanogenic jojoba seeds by various desert rodents, while Compton et al. (1983) observed in a field study that cyanogenic phenotypes of *L. corniculatus* were more frequent in areas of high lemming (*Lemmus lemmus*) densities in Norway and suggested a link between the dynamics of fluctuating rodent populations and changes in the proportions of cyanogenic *Lotus* plants.

In order to investigate this hypothesis, we took advantage of the fact that fossorial *Arvicola terrestris* is a highly selective and destructive herbivore that feeds preferentially on *T. repens* under both field and experimental conditions (Kopp, 1988, 1993). Different from invertebrates, whose major impact might be restricted to early developmental plant stages (e.g., on seedlings or germinating plants), voles are likely to destroy well-established plants over wide areas. In addition, populations of this rodent show strong fluctuations in numbers with periods of high and low densities recurring every five to eight years (Saucy, 1988, 1994). Furthermore, during a field study encompassing the duration of such a population cycle, corresponding cyclic changes in the abundance of *T. repens* have been recorded by Kopp (1993), who suggested that cyanogenesis could be involved in this process.

In this paper, we report results of tests conducted in the laboratory with two agronomic *T. repens* varieties (Ladino and Aran) differing strongly in their cyanogenic properties. Our goals were to determine whether voles are able to discriminate between cyanogenic and acyanogenic plants, and whether they are deterred from feeding on the former. In addition, we conducted complementary experiments with the same cultivars and slugs of the genus *Arion*.

METHODS AND MATERIALS

In order to investigate the ability of fossorial *A. terrestris* to distinguish between cyanogenic (CN^+) and acyanogenic (CN^-) plants, we performed two series of experiments, one conducted in cages and the other in outdoor enclosures. In both series of tests, animals were offered a choice between two white clover varieties: the weakly cyanogenic variety Ladino (L; origin: plain of the Po river, Lodi, Italy) and the strongly cyanogenic variety Aran (A; Oak Park Centre, Carlow, Ireland). These varieties are known from the literature for their extreme quantitative differences in cyanogenic glycosides, the ratio of Ladino/Aran for cyanogenic glycosides being approximately 1/100 (cyanide content: 26 and 2405 mg HCN/kg of dry matter, respectively) (Lehmann et al., 1991), while the percentage of cyanogenic individual plants in these varieties is less than 2% in Ladino and higher than 98% in Aran (Caradus, 1986; Williams, 1987). Both Ladino and Aran are large-leafed varieties and are phenologically very similar.

Plants were grown from seeds sown in the spring in rectangular flowerpots (cage experiments) or in square outdoor tanks (outdoor enclosure experiments) and voles were allowed to feed on live plants. In both experiments, the containers were divided into four subunits. Seeds of each of the two varieties were sown in two of these subunits according to a 2×2 alternate design, with plants of the same variety sown in opposite positions (i.e., in the top right and bottom left subunits and reciprocally). In addition, the disposition of the CN^+/CN^- varieties was random-

ized among pots. Experiments were started when the plants had grown in a regular and uniform canopy. Because these varieties are large-leafed and phenologically very similar, it was impossible (at least for us) to detect any pattern indicating the sowing design. It is therefore reasonable to assume that the voles could not rely on visual cues when they selected to feed in a particular plot.

Cage Tests in the Laboratory. For these experiments, we used the Ladino California cultivar, which is commonly included in many seed mixtures, and the cyanogenic Aran variety. In April 1994, plants were sown in heat-sterilized compost and grown in rectangular 40 × 30 × 10-cm flowerpots for about three months in a greenhouse. Plants were mowed after three weeks in order to stimulate vegetative growth. To avoid having the voles dig into the flowerpots during the tests and destroy the whole experimental set up, pots were covered at sowing time with a 10 × 10-mm wire netting (diameter 0.5 mm) and plants were allowed to grow in the interstices. Physical separations between the four sowing subunits of each flowerpot allowed us to accurately harvest each of the two white clover varieties.

The cage tests were performed between July 29, 1994, and August 19, 1994. Field-caught voles kept in captivity for four to five weeks were placed in 40 × 50 × 40-cm wire cages in which the experimental flowerpots were lying on the floor. The 5-cm-wide spaces between the flowerpots and the opaque walls of the cages were covered with a wooden lid, which created a system of runways mimicking a burrow system. Four openings in the lids (one on each side of the cage) allowed the voles to reach the plants. Voles readily adopted this set-up and built their nests below the lids. Preliminary tests showed that the voles consumed approximately 50% of the offered food within two days, and the tests were therefore run for 48 hr. No water or alternative food was provided.

Data Analysis. By offering living plants to the animals, there was no nondestructive way of getting direct estimates of the quantity of each variety offered to the voles before the tests and, therefore, of getting estimates of the quantity of food actually eaten in each particular test. For this reason, we used an indirect approach. We decided to compare estimates of the remaining dry matter of both varieties at the end of the tests against a series of control pots. In this approach, preferences can be expressed as ratios between the acyanogenic and cyanogenic varieties, i.e., Ladino over Aran (L/A or CN^-/CN^+) with smaller or larger ratios in the experimental setting than in the control tests indicating a preference for the acyanogenic or the cyanogenic variety, respectively. Statistically significant differences between control and experimental tests were detected with the nonparametric Mann-Whitney test, and one-tailed comparisons were performed because we expected a preference for the acyanogenic variety. Therefore, at the end of the tests, the vegetation remaining in experimental pots was cut (separately for each variety) and dried overnight in an oven at 55°C. Control pots were treated in a similar way. Cyanide contents of both varieties were estimated from preserved

samples from the control pots. These analyses were performed in the laboratory of the Swiss Federal Station for Animal Production, CH-1725 Posieux, by the distillation method described by Pulss (1962).

Tests in Outdoor Enclosures. In addition to the laboratory cage tests, complementary experiments were conducted in outdoor enclosures in which voles were allowed to dig underground burrows, to feed, and to behave in their usual way. Sixteen polyester tanks ($2 \times 2 \times 0.5$ m) arranged in a 4×4 design were built and placed on the ground at the perimeter of the university campus. They were filled with a bed of 35 cm of earth from a neighboring natural grassland. The tanks were covered with "vole-proof" lids to restrain the animals from escaping, as well as to protect them from predators. Small holes (1 cm diam.) in the bottom of the tanks allowed for water drainage.

In June 1994, two varieties of *Trifolium repens* (Ladino and Aran) were sown according to the 2×2 alternate design described earlier in four contiguous 0.8×0.8-m squares located in the center of the tanks. In order to minimize edge effects along the walls of the tanks, the remaining 20-cm-wide strip was sown with rye grass (*Lolium perenne;* a grass well accepted by fossorial *A. terrestris* when dicots are absent) (Kopp, 1993). In this case, however, Ladino California was replaced by the even less cyanogenic Ladino Espanso (Lodi, Italy; <10 mg HCN/kg dry matter) (Lehmann et al., 1991), after it was realized that the Californian cultivar had been imperfectly controlled by the breeder for its content in cyanoglucosides, as compared to the original Italian variety.

In July 1994, three weeks before the beginning of the experiments, 20 voles (10 males and 10 females) were paired and became accustomed to each other in laboratory cages. In August, they were released in 10 enclosures assigned at random among the 16 tanks. The six remaining tanks were used as control units. Experiments were run for periods varying between 11 and 29 days (average: 18 $\pm$ 2 days) depending on the rate at which the voles had consumed approximately half of the available vegetation.

Data were analyzed in a similar way as in the cage experiments and are expressed as ratios of dry matter for both varieties (CN^-/CN^+ ratios). In addition to these measures, we also recorded at two-day intervals the above-ground signs of vole activity in the enclosures (vole hills, holes, feeding signs, etc.). Preserved samples of *T. repens* were also analyzed for their cynanide content.

Tests with Slugs. In addition, we conducted tests with slugs of the genus *Arion* (*A. ater* and *A. subfuscus*) in order to determine whether these invertebrates, which had been widely used in early demonstrations of the role of cyanogenesis (Crawford-Sidebotham, 1972; Jones, 1988), would support our observations with voles. Plants were grown from seeds in $25 \times 25 \times 12$-cm pots in spring 1996, either in pure Ladino Espanso or Aran stands or in mixtures (according to a 2×2 alternate design in the latter case) with eight replications of each of the three treatments. After germination, the number of plants per pot was adjusted

to 20 and the plants were allowed to grow for 45 days in the greenhouse. The pots were covered with gauze to protect the plants from herbivores. Three slugs were introduced for five days in each pot (until approximately half of the leaves had been consumed). The pots were regularly watered to keep the slugs alive and active and remained covered to prevent the mollusks from escaping. At the beginning and at the end of the experiments, the number of white clover leaflets were counted and the slugs were weighed.

RESULTS

Tests in Laboratory Cages. The results from tests in laboratory cages indicate that the voles left quantitatively less or ate more of the acyanogenic Ladino variety compared to the cyanogenic Aran (Table 1). On average, the L/A ratio dropped from 0.94 ± 0.08 (mean $\pm$ SD; $N = 11$) in control pots to 0.68 ± 0.23 in experimental trials ($N = 13$ animals tested). Therefore, a clear bias of the CN^-/CN^+ ratio was observed, indicating that the voles ate more of the Ladino variety, the acyanogenic form. The average difference in ratios between control and experimental pots is 0.26 and is significant ($z = -2.926$, $P < 0.01$, Mann-Whitney nonparametric test, one-tailed). The results (Figure 1a) show a scatter of CN^-/CN^+ ratios, ranging between extreme preference for the acyanogenic variety (ratio = 0.23) to lack of discrimination (ratio = 1.0). No indication of a preference for the cyanogenic variety was recorded. The average cyanide content was estimated to be approximately 9 times lower in Ladino California (160 ± 42 mg HCN/kg of dry matter; $N = 8$) than in Aran (1416 ± 120 mg/kg; $N = 10$; Table 1).

Tests in Outdoor Enclosures. In outdoor enclosures, the results were similar and consistent with those gathered in the cage experiments (Table 1). On average, the experiments lasted for 18 days. In this case, the average CN^-/CN^+ ratio was 1.17 ± 0.16 and 0.89 ± 0.24 for control and experimental trials, respectively. Therefore, the voles also showed a bias in favor of acyanogenic white clover in the outdoor enclosures, with an average difference in ratios of 0.28 (very close to the difference of 0.26 in the cage experiment). As in the cage tests, this difference is significant ($z = -3.322$, $P < 0.02$, Mann-Whitney, one-tailed). As a whole, CN^-/CN^+ ratios ranged from 0.5 to 1.3 for experimental trials and from 1.0 to 1.4 for controls (Figure 1b). Eight of the 10 pairs of voles showed a preference for the acyanogenic form, while two pairs did not. However, no pair showed a preference for the cyanogenic form. As a main difference with the cage tests, Ladino Espanso was significantly more productive than Aran in the outdoor enclosures.

The cyanide content was estimated to be 1369 ± 140 mg/kg for Aran ($N = 6$). For the Ladino Espanso variety, three of six samples tested were below the detection level of the method, and the three remaining samples showed a level of 14 ± 3 mg HCN/kg dry matter (Table 1).

TABLE 1. RESULTS OF CHOICE TESTS IN LABORATORY CAGES AND OUTDOOR ENCLOSURES WITH *Arvicola terrestris* AND LADINO (ACYANOGENIC) AND ARAN (CYANOGENIC) VARIETIES OF *Trifolium repens*[a]

Variety	Average dry matter (g ± SD)		Average diff. (C − E) (g)	Average HCN content [mg/kg (mean ± SD)]	Estimated HCN intake (mg/indiv/day)
	Control group	Experimental group			
Laboratory cage experiments	(N = 11)	(N = 13)			
Ladino (California)	18.6 ± 4.99	7.9 ± 5.83	10.7	160 ± 42 (N = 8)	0.86
Aran	19.8 ± 5.28	11.0 ± 6.96	8.8	1416 ± 120 (N = 10)	6.23
Ratio L/A	0.94 ± 0.08	0.68 ± 0.23	0.26		
Outdoor enclosure experiments	(N = 6)	(N = 10)		(N = 6)	
Ladino (Espanso)*	219.9 ± 67.0	113.1 ± 29.4	106.8	14 ± 3	0.04
Aran	175.6 ± 36.8	133.7 ± 39.9	41.9	1369 ± 140	1.59
Lolium	451.0 ± 56.4	327.9 ± 70.3	123.1		
Ratio L/A	1.17 ± 0.16	0.89 ± 0.24	0.28		

[a]Average dry matter: average dry matter (g) harvested for each variety at the end of the experiments. Average of 11 and 13 cases in cage experiments and of 6 and 10 experimental units in outdoor tanks for control and experimental groups, respectively. Average diff. (C-E): average difference in harvested dry matter (g) between the control and the experimental groups. Average HCN content: average content in cyanoglucosides for both varieties expressed as mg HCN/kg of DM. Estimated HCN intake: estimates of the daily intake in cyanoglucosides per individual (expressed in equivalents of mg HCN/kg of body mass).

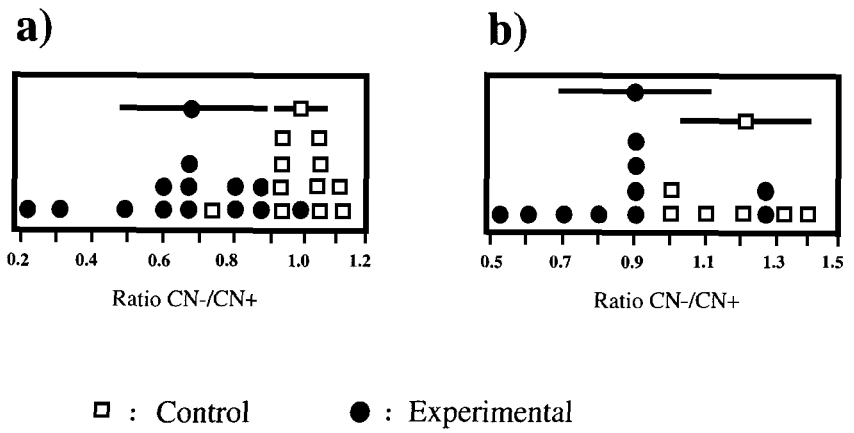

FIG. 1. Detailed results of the tests conducted with the vole *Arvicola terrestris* and the two varieties of *Trifolium repens*. Black dots and white squares indicate results of individual observations for experimental and control trials, respectively, in (a) choice tests conducted in laboratory cages and (b) in outdoor enclosures. Average values with standard deviations are indicated on top of the graphs.

Tests with Slugs. As shown in Table 2, slugs also preferred the acyanogenic Ladino variety. At the beginning of the tests, each of the 20 plants in a pot had produced approximately four leaves or 12 leaflets, corresponding to an average of about 240 leaflets per pot. When the plants were offered in pure stands, the slugs ate significantly more of the Ladino (CN^-) variety (54% or 138 of 256 leaflets on average), as compared to 35% (83 of 233) of the Aran cyanogenic morph (Table 2; $z = -2.896$, $P < 0.01$, one-tailed Mann-Whitney test). Slugs were even more selective when the plants were offered in mixed stands. On average, they ate 71% and 33% of each variety, respectively (Table 2; $z = -3.046$, $P < 0.01$, one-tailed Mann-Whitney test). In addition, it was possible with mixed stands to perform statistical tests for each individual trial, and differences were found in seven of the eight cases (Table 2; $P < 0.01$; χ^2). Figure 2 also shows that the slugs ate significantly more (Figure 2a) and increased more in body mass (Figure 2b) when they were allowed to feed on acyanogenic plants.

DISCUSSION

The results of these experiments with two varieties of *Trifolium repens* (Ladino and Aran) experimentally confirm and extend data from vole and plant species from previous observations reported by Jones (1962, 1966) for *Microtus agrestis* and *Lotus corniculatus*. In contrast with the earlier studies, we tested

TABLE 2. RESULTS OF CHOICE TESTS CONDUCTED IN LABORATORY WITH SLUGS (*Arion* sp.) AND LADINO ESPANSO (ACYANOGENIC) AND ARAN (CYANOGENIC) VARIETIES OF *Trifolium repens*[a]

	Number of leaflets at the beginning		Number of leaflets remaining at the end		Number of leaflets eaten			
	Aran (CN^+) ($N = 7$)	Ladino (CN^-) ($N = 8$)	Aran (CN^+) ($N = 7$)	Ladino (CN^-) ($N = 8$)	Aran (CN^+) ($N = 7$)	Ladino (CN^-) ($N = 8$)	P	Statistical test used
Pure stands								
Average	233.1	256.5	150.0	118.4	83.1	138.1		1-tailed
Range	210–261	234–261	136–167	73–168	47–125	110–182		Mann-
SD	14.8	14.4	11.5	28.5	22.0	23.5	<0.01	Whitney
Mixtures of Ladino and Aran								
Trial 1	144	120	90	35	41	72	<0.01	χ^2
Trial 2	108	114	78	16	25	93	<0.01	χ^2
Trial 3	111	114	75	39	33	72	<0.01	χ^2
Trial 4	108	123	78	42	30	81	<0.01	χ^2
Trial 5	129	129	75	40	51	86	<0.01	χ^2
Trial 6	84	96	68	15	13	78	<0.01	χ^2
Trial 7	111	93	69	46	27	32	>0.05	χ^2
Trial 8	114	126	79	32	26	85	<0.01	χ^2
								1-tailed
Average	113.6	114.4	76.5	33.1	30.8	74.9		Mann-
SD	16.3	12.5	6.4	10.9	11.4	18.7	<0.01	Whitney

[a]Global results of the experiments with 20 plants sown in pure stands and detailed results of the eight trials for tests with plants sown in mixtures of the two varieties (10 Aran and 10 Ladino plants). The number of leaflets present at the beginning and at the end of the tests are given for the two varieties. The number of leaflets eaten has been calculated under the assumption that the plants did not produce new leaves during the course of the experiments. Number of eaten leaflets = number at the beginning − number at the end − number wasted.

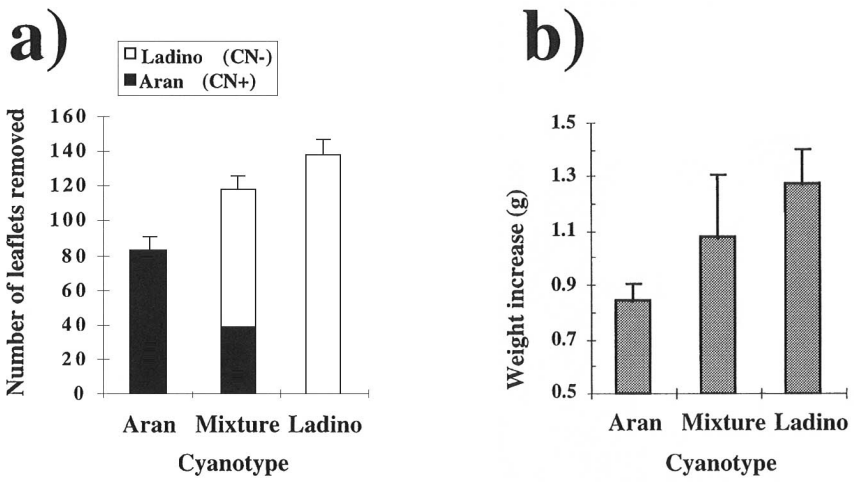

FIG. 2. Results of the choice tests conducted with slugs (*Arion* sp.) and the two varieties of *Trifolium repens*. (a) Average slug consumption in pure and mixed stands, (b) average individual change in slug body mass. Vertical bars indicate standard errors.

a larger number of individual voles (total = 33 in 23 experimental units, as compared to two individuals in the same pit, or one experimental unit for *M. agrestis*). Furthermore, our results have been replicated in two different experiments, one of which attempted to mimic natural field conditions. Our results indicate that fossorial *A. terrestris* are able to distinguish between two varieties of white clover, presumably on the basis of their differences in cyanogenic glycoside content. Complementary experiments with slugs of the genus *Arion* also confirm the well-established results obtained by various authors more than 20 years ago (Jones, 1988, 1998).

Comparisons between control and experimental tests, however, also show that voles ate substantial amounts of the cyanogenic morph, a result that has been reported in many studies both in mammals and in invertebrates (Jones, 1998). If we estimate the food consumed as the difference $(C - E)$ between control (C) and experimental trials (E) for both forms and the relative proportion as $(C-E)/C$, voles ate on average 57.6% and 44.4% of the acyanogenic Ladino and the cyanogenic Aran standing crops, respectively. It is also worth mentioning that in several cases, we noted that the voles only ate the leaves of the plants, leaving the stems untouched. This always affected the acyanogenic Ladino variety (in *T. repens* cyanogenic glycosides are concentrated in the leaves). Therefore, the differences in consumption reported above may have been partly underestimated.

During our two-day laboratory cage experiments, the voles (weighing 70.8 ± 2.3 g on average at the beginning of the experiment) ate on average, 19.8 g

of dry matter, or 66 g of fresh food per day. This amount corresponds approximately to 93% of their body mass, which is in the range of what is known for the physiology of this animal (Grenot et al., 1984; Kopp, 1993), especially if we take into account that part of the food was wasted. We estimated that the voles consumed on average 7.1 mg of cyanide per day which corresponds to a dose of 101 mg HCN/day/kg body mass. No animal died during the following days or weeks, and they maintained constant body weights during the experiment (average weight change = 0.1 ± 2.0 g, ranging from −3 to +4 g). By comparison, the lethal dose of KCN when taken orally in a single dose is 3–5 mg/kg in humans (Seigler, 1991). The LD_{50} of KCN in mice is 2–6 mg/kg (intraperitoneal injections) and approximately three times higher in the rat (Seigler, 1991; Speijers, 1993). Although our animals did not ingest pure cyanide, these results indicate that the absorbed doses are, by all standards, high for mammals. It should be remembered, however, that mammals can detoxify large amounts of cyanogenic glycosides when they are ingested in small quantities over long periods of time (Jones, 1998).

Applying the same line of reasoning for outdoor experiments, we estimated that each pair of voles consumed on average 106.8 g dry matter of the Ladino variety (approximately 48.6% of the available acyanogenic biomass) and only 41.9 g dry matter of the Aran variety (23.9% of the cyanogenic biomass). Thus, they ate approximately 2.5 times more of the nontoxic form of white clover. They also consumed 123 g dry matter of *Lolium perenne* (27.3% of the grass standing crop). We estimated the vole individual daily consumption to be approximately 63 g of fresh food per day. Cyanogenic plants accounted for only 15.4% of the total ingested biomass, as compared with 44% in the cage experiments. On average, the voles ingested of 1.6 mg HCN/individual/day, which corresponds to approximately 25 mg HCN/kg body mass.

In contrast to the results of the cage experiments, in this case voles were in poor condition at the end of the experimental period. Interestingly, one individual was not recaptured and probably died in the tank, while two voles died in the animal house within a few days after having been removed from the enclosures. Moreover, 14 of the 19 individuals lost weight (between 12 and 23 g for six of them). The average change in body mass was −6.4 ± 2.0 g and was significant statistically ($t = -3.305$, $P < 0.01$, two-tailed t test, $df = 18$). Because it may be argued that animals from the same tanks do not correspond to independent units, we made the same calculations by using the average difference in body mass for the pairs in each enclosure with similar results (average difference: −6.3 ± 2.6 g; extremes: −20.5 and +3 g; $t = -2.561$, $P < 0.05$; two tailed t test; $df = 9$).

The results of the experiment in outdoor enclosures suggest that, apart from being in poor condition, fossorial *A. terrestris* significantly reduced their total consumption of the toxic morph from 101 mg HCN/day/kg during the 48-hr tests to a level of approximately 25 mg HCN/day/kg during experiments lasting

for 18 days. Therefore, voles ingested amounts of cyanide that were in the upper range of what is still considered as sustainable for many mammals (Jones, 1998), but can cause serious reproductive trouble in sheep and cattle (Lehmann et al., 1991; Gutzwiller, 1993). This reduction in cyanogenic white clover consumption indicates that voles might have progressively adapted their diet in reducing its content in cyanoglycosides, while the important weight losses recorded during the study, as well as the deaths, suggest that voles may have been intoxicated by high amounts of cyanide intake, a point that would be worth investigating further.

Both laboratory cage and outdoor enclosure experiments indicate that when given the choice, fossorial *Arvicola terrestris* are able to discriminate between cyanogenic and acyanogenic forms of white clover and prefer the latter. It could be objected that in our experiments we cannot exclude that the voles preferred the Ladino morph for reasons unrelated to its low cyanic content. In order to exclude this possibility, we should have worked with different selected cyanotypes. For logistic and practical reasons and because the amount of material needed for the tests was so large (tens of square meters) and exceeded by far our cultivation and breeding capacities, we decided to work with two varieties differing strongly in their cyanide contents. As a complementary approach, we have extended our experiments to six varieties using a different experimental approach. The results, which will be reported in a future publication (Viette et al. in preparation), fully confirm that voles prefer the less cyanogenic cultivar. The fact that slugs, which are unrelated organisms and which have long been shown to be very sensitive to cyanogenic glycosides, also preferred the acyanogenic variety, can be considered as indirect evidence in support of our interpretation that the preference recorded for the Ladino cultivar is mainly related to its low content in cyanogenic glycosides.

In conclusion, in this paper we confirm and extend to the vole *Arvicola terrestris* and to the plant *Trifolium repens* early observations of Jones (1962, 1966) on *Microtus agrestis* and *Lotus corniculatus*. We show that fossorial *Arvicola terrestris* feed preferentially on acyanogenic plants when given the choice. Our observations also suggest that these voles seem able to adapt their diet and cyanide intake to sustainable levels, which are similar to those reported for several other mammal species.

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