Ann. Anim. Sci., Vol. 15, No. 1 (2015) 119–127 *DOI: 10.2478/aoas-2014-0060*

DE GRUYTER
OPEN

EFFECT OF *ACHILLEA MILLEFOLIUM* L. AND *MATRICARIA CHAMOMILLA* L. ON CONSUMPTION OF *JUNIPERUS OXYCEDRUS* L. AND *J. PHOENICEA* L. BY GOATS*

Jozo Rogosic[1], Tomislav Saric[1], Ivan Zupan[1♦], Kevin Welch[2]

[1]Department of Ecology, Agronomy and Aquaculture, University of Zadar, Trg Kneza Viseslava 9, 23 000 Zadar, Croatia
[2]Poisonous Plant Research Laboratory, USDA, Logan, UT 84341, USA
♦Corresponding author: zupan@unizd.hr

Abstract

Reduction in herbage production and plant diversity on rangelands on the coastal part of Croatia, due to invasion of terpene-rich shrubs has been recorded during the recent period. Therefore, there is a strong need to understand ecological relationships between plant and animal components of these shrubby ecosystems. Through four consecutive experiments, we investigated the potential of two well-known medicinal herbs, *Achillea millefolium* L. (yarrow) and *Matricaria chamomilla* L. (chamomile), as a feed supplement to increase intake of two terpenoid shrubs, *Juniperus oxycedrus* and *J. phoenicea*, by goats. Preliminary research indicated that both yarrow and chamomile had a positive influence on the intake of *J. phoenicea* by goats, but not on the consumption of *J. oxycedrus*. Our model could be implemented in the field as an approach to reduce the abundance of *J. phoenicea* in the environment and to improve the quality of herbage (grasses and forbs) on Mediterranean pastures.

Key words: chamomile, medical plants, small ruminants, sustainable livestock production, yarrow

Mediterranean maquis vegetation is characterized by low quality of forage and abundant plant defense but still represents significant forage resource for small ruminants, especially goats (Papachristou et al., 2005; Rogosic et al., 2006 a). Juniper species, including *Juniperus oxycedrus* and *J. phoenicea*, are generally considered unpalatable and are an increasing component of many rangelands in Mediterranean parts of Croatia and Bosnia/Herzegovina, infesting rangelands previously dominated

*This research has been conducted as a part of the project 269-0000000-3597 "Potential mechanisms for better use of forage potential of the Mediterranean maquis" funded by Ministry of Science, Education and Sport, Republic of Croatia.

by grasses (Rogosic, 2000). The dense shade of juniper canopy and deep accumulation of juniper leaf litter under larger trees appear to be the primary constraints to herbaceous species diversity and production on Adriatic pastures, resulting in a loss of perennial grasses and forbs production under the canopy of juniper trees (Smeins et al., 1994). Conventional juniper shrub control methods, such as chaining, tree dozing or grubbing, bulldozing and root plowing are impractical primarily because of the high cost involved (Scifres, 1980; Johnson et al., 1999).

Sheep and goats normally do not utilize juniper species due to a high concentration of terpenoid compounds that are responsible for aversive post ingestive feedback (Provenza, 1995; Villalba and Provenza, 2005; Rogosic et al., 2006 b; 2006 c). Another reason for low palatability of juniper species is the inhibition of rumen and cecal/colon microbes, since reduced activity of microbes in the digestive tract leads to decreased diet digestibility (Nagy et al., 1964; Oh et al., 1967; Schwartz et al., 1980). Several nutritional supplements that might enhance juniper consumption have been proposed so far. Supplementation with vitamins, minerals, amino acids and carbohydrates often enhances the ability of herbivores to detoxify, or tolerate, phytotoxins such as essential oils (Boyd and Campbell, 1983). A protein, rather than energy, supplement appears to be more beneficial to goats consuming juniper (Taylor, 1997). Activated charcoal supplementation resulted in an increased intake of high terpene-containing Mediterranean shrubs when a mixture of three shrubs (*J. phoenicea*, *Helichrysum italicum*, and *J. oxycedrus*), two shrubs (*J. phoenicea* and *H. italicum*) or one shrub (*J. phoenicea*) species was offered (Rogosic et al., 2006 c). Similarly an increased intake of *J. phoenicea* was recorded when sheep were supplemented with barley and Optigen® (Saric et al., 2013).

Yarrow (*Achillea millefolium* L.) is a widely used medicinal plant native to the Northern hemisphere. It has been popular for millennia and the name of the genus originates from the ancient use as a wound-healing remedy by the Greek hero Achilles of the Trojan war, whereas millefolium refers to the deeply divided leaves (Benedek and Kopp, 2007). Yarrow is traditionally used for the treatment of inflammatory and spasmodic gastro-intestinal disorders as well as hepato-biliary complaints. Moreover, it is used as an appetite-enhancing drug, for wound healing and against skin inflammations (Benedek and Kopp, 2007). The pharmacological effects of yarrow are mainly due to the essential oil content (Nemeth and Bernath, 2008).

Chamomile (*Matricaria chamomilla* L., fam. Asteraceae) is one of the important medicinal herbs native to southern and eastern Europe and can be found as an introduced species in many other parts of the world (Singh et al., 2011). Chamomile has been used in herbal remedies for thousands of years to treat flatulence, colic, intermittent fever, disturbance of the stomach associated with pain, sluggish digestion, diarrhea and nausea (Singh et al., 2011). The antispasmodic effect of alcoholic extracts of *M. chamomilla* has been established by Forster et al. (1980). The dried flower heads of chamomile have been reported to exhibit spasmolytic and sedative properties, although the active components responsible for the sedative activity have not yet been fully characterized (Avallone et al., 2000). There is a lack of data about the effects of chamomile as a feed supplement on ruminant health. However, recently it was reported that an extract of *M. chamomilla* showed favorable results as an anthelmintic in goats and sheep (Bahrami et al., 2010, 2011).

There is little known regarding the effects of medicinal plants on the intake of naturally occurring plants rich in plant secondary metabolites (PSM). Our hypothesis is that offering yarrow (*A. millefolium* L.) and chamomile (*M. chamomilla* L.) to goats can result in an increased intake of toxic shrubs, thus effectively increasing the biological diversity of plant species in diet. Therefore the objective of this study was to determine whether intake of these medicinal herbs by goats increases consumption of the terpene-rich species *J. oxycedrus* L. and *J. phoenicea* L.

Material and methods

Study area

We conducted four trials at the University of Zadar ecological research station located 25 km south of Mostar, in the border area between Bosnia/Herzegovina and Croatia. Both juniper shrub species were hand-harvested within one week, in a 2 km diameter from the research station. Leaves and 1-year old twigs of *J. oxycedrus* and *J. phoenicea* shrubs (about 10 cm in length) were clipped and placed inside paper bags. Within 1 h after collection, the plant material was ground to 1 cm in length with a chipper, mixed for uniformity, placed in woven polyethylene bags and refrigerated at 4ºC. During the experiments, bags with the shrubs to be fed that day were removed from cold storage in the morning and offered immediately to animals. The forb *A. millefolium* was harvested from the surrounding natural meadows every other day, while *M. chamomilla* was harvested every other day from a nearby seeded field. The two harvested forbs were also ground with a chipper, placed in woven polyethylene bags and refrigerated at 4ºC. During the experiments, bags with the forbs were removed from cold storage in the morning and offered to animals in combinations with juniper shrubs.

Shrub chemical constituents and isolation of essential oils

Leaves and 1-year old twigs of *J. oxycedrus* and *J. phoenicea* were analyzed for essential oil content at the Laboratory of Chemistry, Faculty of Natural Science, University of Mostar. Essential oils were isolated by steam distillation using a Clevenger-type apparatus according to the method outlined by the European Pharmacopoeia (2002). The plant materials (100 g fresh weight) were processed by steam distillation for 4 hours.

The main chemical constituents of yarrow essential oil are proazulenes and other sesquiterpene lactones, dicaffeoylquinic acids and flavonoids (Nemeth and Bernath, 2008). A total of 21 compounds were identified in the oil from *Matricaria* using gas chromatography/mass spectrometry (GC/MS), accounting for 92.86% of the oil composition. The main compounds identified were alpha-bisabolol (56.86%), trans-trans-farnesol (15.64%), cis-beta-farnesene (7.12%), guaiazulene (4.24%), alpha-cubebene (2.69%), alpha-bisabolol oxide A (2.19%) and chamazulene (2.18%) (Tolouee et al., 2010).

Animals and experimental protocol

The experimental animals (n = 12; mean weight ± SEM = 12.6 ± 1.1 kg) were 4–5 month old goats (a mixture of domestic breed crossed with Saanen and Alpine breeds). All experimental animals were raised on the same shrublands and were experienced in grazing on Mediterranean maquis vegetation, including juniper species and chamomile. Prior to the experiment animals went through a 5 day pre-conditioning period by adding 200 g of barley mixed with 10 g of every shrub used in experiments. Animals were divided into two groups of six animals (control and treatment). Four separate experiments were conducted, wherein the animals were offered the various diets daily for 10 days. At 8:00, in all experiments animals from both groups were fed with 200 g of barley.

In the first experiment, we compared intake of *J. oxycedrus* alone (control group) with intake of *J. oxycedrus* and *A. millefolium* (treatment group) offered at the same time in separated feed boxes. Animals in both groups (control and treatment) were offered 200 g of *J. oxycedrus* in food boxes from 09:00 to 14:00 h. Animals and food boxes were checked every 30 min and additional *J. oxycedrus* was added as needed. Animals in the treatment group also received 200 g *A. millefolium*, divided in four 50 g meals offered at 9:00, 10:15, 11:30, and 12:45 h. The same protocol was used in the following experiments, but *J. oxycedrus* was altered with *J. phoenicea* and *A. millefolium* with *M. chamomilla*.

In the second experiment, the animals in the treatment group were offered *J. oxycedrus* and *M. chamomilla*, while control animals were only fed with *J. oxycedrus*.

In the third experiment, the control group of goats was offered *J. phoenicea* alone, while treatment group of goats was fed a *J. phoenicea* and *A. millefolium* mixture.

In the fourth experiment, the control group of animals was fed only *J. phoenicea*, while the treatment group of animals was fed *J. phoenicea* and *M. chamomilla*.

Statistical analyses

The experimental design for the shrubs fed individually to goats was a completely random design with a separate analysis for each shrub. The model included two treatments (*A. millefolium/M. chamomilla* + shrub and shrub alone), with individual animals nested within treatments, and repeated measures over the 10-days. The total daily amount consumed of each shrub offered was used as the dependent variable in the analysis. Analyses were conducted using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC; Version 9.1 for Windows). All analyses on shrub intake were adjusted for body weight prior to the analysis (g/kg BW).

Results

The yield of essential oil in *J. phoenicea* shrub was approximately two times higher than in *J. oxycedrus* shrub (0.34 ± 0.02 vs. 0.18 ± 0.03; P<0.01).

In the first experiment, after 10 days of feeding there was no significant difference (P=0.83) in *J. oxycedrus* shrub intake between control (*J. oxycedrus*; 20.16±0.86 g/kg BW) and treatment (*J. oxycedrus* + *A. millefolium*; 19.27±0.64 g/ /kg BW) groups (Figure 1).

In the second experiment, there was no significant difference (P=0.98) in *J. oxycedrus* shrub intake between control (*J. oxycedrus*; 21.37±0.56 g/kg BW) and treatment (*J. oxycedrus* + *M. chamomilla*; 21.13±1.58 g/kg BW) groups (Figure 1).

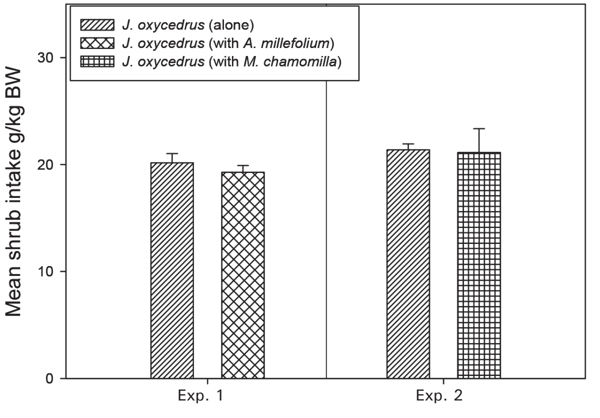

Figure 1. Mean intake ± SEM (g/kg BW) of *J. oxycedrus* in control and treatment groups of goats (n = 6 per group). In both experiments the control goats were fed *J. oxycedrus* alone. In Exp. 1 the treatment group was fed *J. oxycedrus* and *A. millefolium*. In Exp. 2 the treatment group was fed *J. oxycedrus* and *M. chamomilla*. Each experiment lasted 10 days

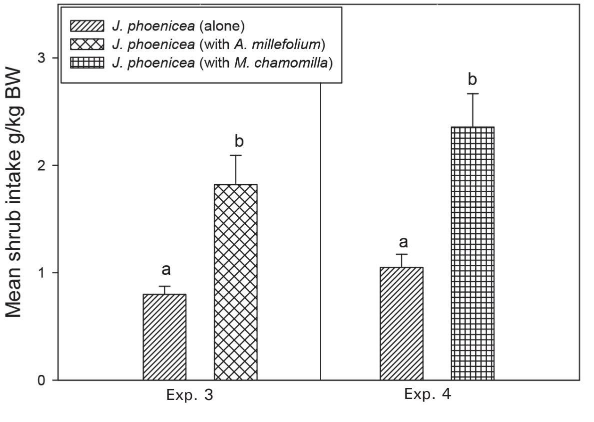

Figure 2. Mean intake ± SEM (g/kg BW) of *J. phoenicea* in control and treatment groups of goats (n = 6 per group). In both experiments the control goats were fed *J. phoenicea* alone. In Exp. 3 the treatment group was fed *J. phoenicea* and *A. millefolium* and in Exp. 4 the treatment group was fed *J. phoenicea* and *M. chamomilla*. Each experiment lasted 10 days. Different letters indicate significant (P<0.05) differences between groups

However, in the third experiment, a significant difference (P=0.007) of *J. phoenicea* shrub intake was noticed between control (*J. phoenicea*; 0.79±0.07 g/kg BW) and treatment (*J. phoenicea* + *A. millefolium*; 2.12±0.31 g/kg BW) groups (Figure 2). Significant differences between control (*J. phoenicea* offered alone) and treatment (*J. phoenicea* + *A. millefolium*) groups of animals were observed on days two, six, seven, eight, nine and ten (data not shown).

In the fourth experiment, a significant difference (P=0.036) was recorded for *J. phoenicea* intake between control (*J. phoenicea*; 1.05±0.12 g/kg BW) and treatment (*J. phoenicea* + *M. chamomilla*; 2.36±0.31 g/kg BW) groups (Figure 2). Significant differences between control and treatment groups of goats were observed on days three, fourth, seven, nine and ten (data not shown).

Intake of *A. millefolium* was similar when it was mixed with *J. oxycedrus* (first experiment) or *J. phoenicea* (third experiment) (P=0.88; Table 1). Also, the intake of *M. chamomilla* was similar when it was mixed with *J. oxycedrus* (second experiment) or *J. phoenicea* (fourth experiment) (P=0.58; Table 1).

Table 1. Intake of medical plants

Exp.	Shrub	Medical plant*	Intake of medical plant (g/kg BW)
1.	*J. oxycedrus*	*A. millefolium*	5.81±0.57
2.	*J. oxycedrus*	*M. chamomilla*	10.99±0.99
3.	*J. phoenicea*	*A. millefolium*	5.66±0.72
4.	*J. phoenicea*	*M. chamomilla*	11.35±1.30

*Consumed along with offered shrub.
Values represent mean intake ± SEM (g/kg BW) (n = 6 goats per group), each experiment lasted 10 days.

Intake of juniper in the control groups in the first and second experiments (20.16±0.86 and 21.37±0.56 g/kg BW), fed only *J. oxycedrus*, was significantly higher (P<0.01) than intake of the control groups in the third and fourth experiments fed only *J. phoenicea* (0.79±0.07 and 1.05±0.12 g/kg BW).

Discussion

When we compared the intake of juniper in the control groups in the first and second experiments, with intake of the control groups in the third and fourth experiments, we found that the animals consumed much more *J. oxycedrus* than *J. phoenicea*. It is well known that *Juniperus* spp. contain terpenes and other secondary metabolites like tannins (Rogosic et al., 2006 b; Achak and Romane, 2009) that restrict their intake and negatively affect the health of the goats (Riddle et al., 1996). Derwich et al. (2010) in a comparative study determined that α-pinene is the main component of the essential oil in *J. phoenicea* and *J. oxycedrus*, while its ratio in *J. oxycedrus* is 31.25%, and in *J. phoenicea* is 49.15% of total essential oil content. They also found different concentrations of other specific terpenes in the essential oil fraction from *J. phoenicea* and *J. oxycedrus*. Moreover, in our research we discovered that *J. phoenicea* contains twice as much essential oil as *J. oxycedrus*. We speculate that the greater proportion of essential oils, together with differences in its composition, is the main reason for the increased intake of *J. oxycedrus* compared to *J. phoenicea* (approximately 20 times higher) in control groups in our experiments. The types of terpenes present in a plant, and their respective concentrations, probably influence the degree of illness that animals experience after consuming terpene-containing plants. Postingestive feedback is an elementary determinant of food preference and intake in ruminants (Provenza, 1995) and it is known that herbivores limit intake of plants to minimize potentially toxic effect of terpenes (Dziba et al., 2006). It is important to stress that the plants offered to the animals in this study were finely milled. Consequently, any mechanical defenses against herbivory, which is important for *J. oxycedrus*, were compromised, while the chemical defenses, which are particularly important for *J. phoenicaea* (Rogosic et al., 2006 b) largely remained intact.

In our study, concomitant feeding of goats with *A. millefolium* and *M. chamomilla* had a positive influence on the intake of *J. phoenicea* (Figure 2) but not on the intake of *J. oxycedrus* (Figure 1). It is interesting to note that the intake of yarrow and chamomile did not differ between the groups fed the two different juniper species, suggesting that the goats did not consume more of the medicinal forbs to compensate for the difference in palatability between the two juniper species. Perhaps the secondary compounds in yarrow and chamomile limit their intakes and therefore intakes were very similar in experimental groups, regardless of the consumption of the remaining forages. Although the amount of PSM from yarrow and chamomile consumed was similar in experimental groups between experiments, their positive effects on food intake are expressed only in combination with *J. phoenicea*. We can speculate that the difference between influence of these medicinal plants on the intake of *J. phoenicea* and *J. oxycedrus* is due to the amount, and composition, of the essential oils in *J. phoenicea* and *J. oxycedrus*. Although we determined that feeding goats with yarrow and chamomile can increase the intake of *J. phoenicea*, we do not know the pharmacological basis for the increased intake. It is known that medicinal plants can have a positive effect on the health and digestion of goats, but it is also known that the chemical complementarity between the various compounds may also cause an increase in intake (Rogosic et al., 2006 c).

Conclusion

In this research, we demonstrated that supplementing goats with the medicinal plants *A. millefolium* and *M. chamomilla* resulted in an increased consumption of *J. phoenicea*. The results presented in this study demonstrate that incorporating *A. millefolium* and *M. chamomilla* in areas in which *J. phoenicea* dominates, and which are used for browsing by goats, could help to increase the intake of this less palatable plant. This would aid in the development of sustainable livestock production on poor shrublands. Based on these findings future research is needed for improvement of rangeland management in the Mediterranean ecosystems.

References

Achak N., Romane A., Alifriqui M., Adams R.P. (2009). Chemical studies of leaf essential oils of three species of *Juniperus* from Tensift Al Haouz-Marrakech Region (Morocco). J. Essent. Oil Res., 2: 337–341.

Avallone R., Zanoli P., Puia G., Kleinschnitz M., Schreier P., Baraldi M. (2000). Pharmacological profile of apigenin, a flavonoid isolated from *Matricaria chamomilla*. Biochem. Pharmac., 59: 1387–1394.

Bahrami A.M., Doosti A., Moosavi A.B. (2010). Effect of *Matricaria chamomilla* L. plant extraction on experimental infected lamb with *Ostertagia ostertagi* parasits. Int. J. Pharmac., 6: 712–718.

Bahrami A.M., Doosti A., Noorian A.M., Bahrami A.M., Mousavi A., Mousavi S.G.R., Modarreskia M. (2011). A study of plant extraction effect on experimental infected goats with *Haemonchus contortus*. J. Med. Sci., 11: 39–45.

Benedek B., Kopp B. (2007). *Achillea millefolium* L. s. l. revisited: Recent findings confirm the traditional use. Wien Med. Wochenschr., 157: 312–314.

Boyd J.N., Campbell T.C. (1983). Impact of nutrition on detoxication. In: Biological basis of detoxication, Caldwell J., Jakoby W.B. (eds). Academic Press, New York, USA, pp. 287–306.

Derwich E., Benziane Z., Boukir A. (2010). Chemical composition of leaf essential oil of *Juniperus phoenicea* and evaluation of its antibacterial activity. Int. J. Agr. Biol., 12: 199–204.

Dziba L.E., Hall J.O., Provenza F.D. (2006). Feeding behavior of lambs in relation to kinetics of 1,8–cineole dosed intravenously or into the rumen. J. Chem. Ecol., 32: 391–408.

European Pharmacopoeia (2002). Fourth edition, Strasbourg, France.

Forster H.B., Niklas H., Lutz S. (1980). Antispasmodic effects of some medicinal plants. Planta Medica, 40: 309–319.

Johnson P., Gerbolini A., Ethridge D., Britton C., Ueckert D. (1999). Economics of redberry juniper control in the Texas rolling plains. J. Range Manag., 52: 569–574.

Nagy J.G., Steinhoff H.W., Ward G.M. (1964). Effects of essential oils of sagebrush on deer rumen microbial function. J. Wildl. Manage., 28: 785–790.

Nemeth E., Bernath J. (2008). Biological activities of yarrow species (*Achillea* spp.). Curr. Pharm. Des., 14: 3151–3167.

Oh H.K., Sakai T., Jones M.B., Longhurst W.M. (1967). Effect of various essential oils isolated from douglas needles upon sheep and deer rumen microbial activity. Appl. Microbiol., 15: 777–784.

Papachristou T., Dziba L., Provenza F. (2005). Foraging ecology of goats and sheep on wooded rangelands. Small Rum. Res., 59: 141–156.

Provenza F.D. (1995). Tracking variable environments: There is more than one kind of memory. J. Chem. Ecol., 21: 911–923.

Provenza F.D., Scott C.B., Phy T.S., Lynch J.J. (1996). Preference of sheep for foods varying in flavors and nutrients. J. Anim. Sci., 74: 2355–2361.

Riddle R.R., Taylor C.A., Kothmann M.M., Huston J.E. (1996). Volatile oil contents of ashe and redberry juniper and its relationship to preference by Angora and Spanish goats. J. Range Manage., 49: 35–41.

Rogosic J. (2000). Gospodarenje prirodnim mediteranskim resursima (in Croatian). Mostar, Bosnia and Herzegovina, Skolska naklada, 352 pp.

Rogosic J., Estell R.E., Skobic D., Martinovic A., Maric S. (2006 a). Role of species diversity and secondary compound complementarity on diet selection of Mediterranean shrubs by goats. J. Chem. Ecol., 32: 1279–1287.

Rogosic J., Pfister J.A., Provenza F.D., Grbesa D. (2006 b). Sheep and goat preference for and nutritional value of Mediterranean maquis shrubs. Small Rum. Res., 64: 169–179.

Rogosic J., Pfister J.A., Provenza F.D., Grbesa D. (2006 c). The effect of activated charcoal and number of species offered on intake of Mediterranean shrubs by sheep and goats. App. Anim. Beh. Sci., 98: 183–195.

Saric T., Rogosic J., Tkalcic S., Zupan I., Sikic Z. (2013). Increased intake of *Juniperus phoenicea* L. by supplementation with barley and Optigen® in sheep. Ital. J. Anim. Sci., 12: 320–322.

Schwartz C.C., Nagy J.G., Regelin W.L. (1980). Juniper oil yield, terpenoid concentration, and antimicrobial effects on deer. J. Wildl. Manage., 44: 107–113.
Scifres C.J. (1980). Brush management principles and practices for Texas and the Southwest. Texas A&M University Press. College Station, TX, USA, 360 pp.
Singh O., Khanam Z., Srivastava M.K. (2011). Chamomile (*Matricaria chamomilla* L.): An overview. Pharmacogn. Rev., 5: 82–95.
Smeins F.E., Owens M.K., Fuhlendorf S.D. (1994). Biology and ecology of Ashe (blueberry) juniper, Taylor C.A. (ed). Proc. Juniper Symposium. TAES Tech. Report 94–2, Texas A&M University, College Station, TX, USA, pp. 9–24.
Taylor A.C. (1997). Biological management of brush. Texas A&M University Research Station, Sonora, Texas http://texnat.tamu.edu/library/symposia/brush-sculptors-innovations-for-tailoring-brushy-rangelands-to-enhance-wildlife-habitat-and-recreational-value/biological-management-of-brush/
Tolouee M., Alinezhad S., Saberi R., Eslamifar A., Zad S.J., Jaimand K., Taeb J., Rezaee M.B., Kawacki M., Ghahfarokhi M.S., Abyaneh M.R. (2010). Effect of *Matricaria chamomilla* L. flower essential oil on the growth and ultrastructure of Aspergillus niger van Tieghem. Int. J. Food Microbiol., 139: 127–133.
USDA, NRCS (2010). The PLANTS Database (http://plants.usda.gov, 8 March 2010). National Plant Data Center, Baton Rouge, LA 70874-4490 USA.
Villalba J.J., Provenza F.D. (2005). Foraging in chemically diverse environments: energy, protein, and alternative foods influence ingestion of plant secondary metabolites by lambs. J. Chem. Ecol., 31: 123–138.

Received: 10 IV 2014
Accepted: 14 VII 2014