Investigation of Habitats and Causes of Population Decline of *Euonymus sanguineus* in Tibet

Kuang WANG[1,2△], Junli AN[1,2△], Yi GAO[1,2], Zhen XING[1,2,3,4*], Weilie ZHENG[3,4]

1. College of Resources and Environment, Tibet Agriculture and Animal Husbandry University, Nyingchi 860000, China; 2. National Engineering Research Center for Floriculture, Tibet Characteristic Flower Research and Development Center, Nyingchi 860000, China; 3. National Field Scientific Observation and Research Station of Nyingchi Mountain Forest Ecosystem, Nyingchi 860000, China; 4. Institute of Plateau Ecology, Tibet Agriculture and Animal Husbandry University, Nyingchi 860000, China

Abstract Tibet is one of the core distribution areas of the Chinese endemic plant *Euonymus sanguineus*, but the population decline is obvious. In order to protect and develop the plant resources in Tibet rationally, the ecological environment and growth conditions in Milin County, Nyingchi City, Tibet were investigated by means of sampling method in order to provide the theoretical basis for the protection, introduction, breeding and cultivation of *E. sanguineus*. The results were as follows: (i) The population quantity of *E. sanguineus* was too small and the survival area was too small, which seriously affected the interspecific mating, seed development and regeneration, and greatly reduced the genetic diversity within the population; (ii) The dry and hot river valley climate and human and animal activities inhibited the seed germination and seedling growth of *E. sanguineus*, resulting in very low survival rate of seedlings, and hindering the population expansion of *E. sanguineus*; (iii) The distribution area is located in the Yarlung Tsangpo river valley, and the natural habitat fragmentation has affected the interspecific gene exchange, resulting in the low seed bearing capacity and germination rate of *E. sanguineus*, which is the direct cause of the population decline of *E. sanguineus*; (iv) The litterfall of *E. sanguineus* had autotoxicity, which inhibited the growth of seedlings under the forest, caused slow natural regeneration of the population, and then caused the population of *E. sanguineus* to be unable to spread and develop.

Key words *Euonymus sanguineus*, Habitat, Population decline

1 Introduction

Euonymus sanguineus is a small tree or shrub in the Celastraceae family[1], which is endemic to China. *E. sanguineus* is distributed in Tibet, Yunnan, Sichuan, Gansu, Shaanxi, Henan, Hubei. In Tibet, *E. sanguineus* grows in the edge of fir forest and thickets at an altitude of 2 400 – 3 500 m. It is produced in Jilong, Dingri, Yadong, Motuo, Bomi and Milin, most concentrated in Milin. The branch and leaf structure of *E. sanguineus* is clear, the shape is graceful, and the flower shape is unique. The fruit is small and it is brightly colored, like a pink pendant. In particular, the fruit is like a flower after ripening and cracking, and after autumn, the leaves turn bright crimson, like a graceful girl in red. At the same time, the flower, fruit and leaf of *E. sanguineus* have a long period, with a very high ornamental value. The *E. sanguineus* has strong adaptability to the land conditions, and can purify the air, with very strong resistance. It has the obvious improvement soil and the ecological environment benefit. The widespread planting of *E. sanguineus* can play an important role in the ecological protection and restoration in Tibet, and can enrich the ecological diversity. The root and bark of the genus *Euonymus* contain many kinds of medicinal components, which have great medicinal and health value[2]. Its root and bark contain a variety of insecticidal active ingredients, hard rubber resources[2-5], and seeds have high oil content, up to 30% – 50%. It can be used not only as edible oil, but also as non-grain diesel energy resources[6-7]. It has great development potential in agriculture, daily chemicals, food and industry, all of which make the study of *E. sanguineus* have important significance.

At present, there is no report on the study of *E. sanguineus*, the population of *E. sanguineus* is small, most of them are adult plants, the number of young plants is very small. The mortality of seedlings is very high, so it belongs to the declining population. Therefore, the investigation of its habitat conditions and the analysis of the causes of its population decline can lay a foundation for the selection of the optimal habitat conditions and its species protection, introduction, breeding, cultivation, resource development and garden application.

2 Morphological characteristics of *E. sanguineus*

E. sanguineus is generally 2 – 7 m tall, the young branch is grayish brown or purple brown. The leaf texture is flexible and thin, elliptic, ovate-elliptic or ovate, 4 – 9 cm long and 2 – 4.5 cm wide. The leaf apex is acuminate, and the leaf margin is finely serrate; the petiole is 5 – 10 mm long; cymes axillary, total pedicel of 1.5 – 6 cm long; white or white green flowers, tetramerous; flower disc square; stamens glabrous. Flowering period is May – July, seed-setting period is August – October, and the capsule is

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* Corresponding author. E-mail: 470382094@qq.com.
△These authors contribute equally to the paper.

oblate, pink, about 1 cm in diameter, 4-winged, triangular wings. In the mature stage (September to October), the seeds dehisce and can be counted as red seeds, and the fruits are usually 4-loculed and one seed per locule[1].

3 Overview of the study area

The study area is located along the Yarlung Tsangpo River in Milin County, Nyingchi City, Tibet Autonomous Region, with geographical coordinates of 93°49′ – 93°56′ E, 29°6′ – 29°10′ N. Milin County is the plateau temperate and semi-humid monsoon climate area, and because the warm current from Indian Ocean and the Bay of Bengal passes through the Yarlung Tsangpo River passage, this area forms a special climate of subtropical, temperate and cold zone. The basic climatic conditions of the study area are shown in Table 1[8].

Table 1 Main climatic parameters of the investigation area of *Euonymus sanguineus* in Tibet

Region	Annual sunshine hours//h	Annual Sunshine %	Annual average temperature ℃	Monthly average temperature ℃	Average daily temperature range//℃	Monthly average maximum temperature ℃	Monthly average minimum temperature ℃	≥0℃ cumulative temperature ℃	≥10℃ cumulative temperature ℃	Duration d
Milin	1 718.9	39	8.2	–5.2	11.8	15.4	3.4	3 030	2 180	154

Region	Extreme maximum air temperature ℃	Extreme maximum geotemperature ℃	Extreme minimum air temperature ℃	Extreme minimum geotemperature ℃	Annual precipitation mm	Annual evaporation mm	Annual water deficiency mm	Frost-free period d	First frost mm/dd	Last frost mm/dd
Milin	28.8	69.3	–15.8	–16.1	675.1	1 185.9	46	154	10/14	5/16

4 Research methods

4.1 Sample plot setting and investigation On the basis of comprehensive investigation of the region along the Yarlung Tsangpo River in Milin County, the community distribution of *E. sanguineus* was investigated by means of sample plot investigation method. Four typical vegetation communities were selected in the sample plot, and the basic situation of sample plot was shown in Table 2. Three 100 m × 100 m sample plots were set up in each community. The altitude, slope, aspect, soil type, thickness and disturbance degree of human and animal in the sample plot were recorded by electronic total station, GPS and light intensity instrument. The community point map was drawn, the plant density, tree height, crown width and canopy height of *E. sanguineus* whose DBH was more than 5 cm in each plot. A sample of 20 m × 20 m was randomly set in each plot to investigate the associated species and competitive species.

4.2 Seedling survival statistics The number and distribution range of *E. sanguineus* seedlings, and the number of seedlings after four months were measured in the sample plots, and the survival amount of seedlings was calculated.

4.3 Seed-setting ability investigation Using sample plot statistical method, the *E. sanguineus* plants were selected in each sample plot, camera was used to photograph the sunward surface and the shady surface of the flowering and seed-setting plants, and according to the differences of flower, fruit and leaf color and area, the rate of flowering and seed-setting was estimated. A total of 100 *E. sanguineus* fruits were selected randomly in each sample plot. After the capsule dehisced completely, the number of seeds was counted (for the seeds falling off, the residual locules in capsule can be counted). The seed yield, the number of empty seeds, the number of rotted seeds and the number of full seeds were counted, and the abortive rate, the disease rate, the rate of insect pests and the rate of plumpness were calculated.

4.4 Experiment on the key factors in seedling lethality The pH of soil was measured by digging three soil samples from the sample plot. The seedlings were cultivated under different soil and different light intensity and the growth of seedlings was observed. The litters of *E. sanguineus* were collected, and the influence of litters of *E. sanguineus* on the seedling growth was observed by allelopathic experiment.

5 Results and analysis

5.1 Comparative analysis of different habitat conditions and their impacts

5.1.1 Main climatic parameters in the investigation area. From the main climatic parameters of *E. sanguineus* habitat investigation area in Table 1, it was found that *E. sanguineus* growth area is located in the Yarlung Tsangpo River valley area, where the temperature difference between day and night is big, the precipitation is little, the evaporation is big, and the climate is dry and hot. It is featured by the dry and hot river valley climate conditions. The data showed that the growth condition was very bad, which was very bad for seed germination and seedling survival.

As shown in Table 2, in the investigation of the habitat conditions of the *E. sanguineus* population, it was found that the distribution of the community was mainly concentrated on the south bank of the Yarlung Tsangpo River valley. Its topography is mainly the hillside forest edge and thicket, and the soil types are mainly mountain brown soil, mountain cinnamon soil, and sandy loam soil. In the investigation, it was found that the four communities were distributed far apart, in the form of multiple separated fragments, and the area of each patch was very small. This makes the individuation and differentiation of the communities can not be exchanged and complemented, which is not conducive to the development of the population.

Table 2 Basic information of community habitats

Community	Coordinates	Elevation // m	Slope // °	Aspect	Soil type	Soil thickness // m	Disturbance degree
1	93°56′0.58" E, 29°10′40.75" N	3 036	15	Northwest	Mountain brown soil	0.8	Light
2	93°54′46.10" E, 29°9′32.33" N	2 973	<3	–	Mountain cinnamon soil	0.6	Moderate
3	93°54′26.39" E, 29°9′32.33" N	2 991	20	Northeast	Mountain cinnamon soil	0.6	Moderate
4	93°49′51.59" E, 29°7′9.99" N	3 020	25	Northeast	Mountain brown soil	0.9	Light

5.1.2 Associated and competitive species. As shown in Table 3, according to the investigation, the trees are competitive species, shrubs are competitive and associated species, and grasses and ferns are associated species in the habitat. There was a remarkable feature in all the sites investigated, that is, the plant species began to be rich except for a small number of herbs and ferns in the crown range (with no other plants at all).

From the tables and figures in the article, it was found that with the decrease of associated and competitive species, and the decrease of disturbance by human and animal, the basic characteristic index of the population of *E. sanguineus* increased and developed in a favorable direction. This was related to the improvement and upgrading of the growing environment, as well as the acquired growth resources and space, as a result of the decrease of associated and competitive species. However, the decrease of associated and competitive species, especially the decrease of shrubs, weakened its protection circle and it was easily disturbed by human and animal activities. It is difficult for seeds to germinate, seedlings to survive, and the characters of plant are weak due to the compaction of soil under or near the plant which is seriously disturbed by human and livestock, causing a great impact on the growth and development of the population.

However, during the investigation in many places, due to human and animal disturbance and natural factors, it was found that the natural habitat of *E. sanguineus* presented many separate small fragments, that is, habitat fragmentation. Habitat fragmentation can reduce the total area of the original habitat and produce isolated heterogeneous populations, thus affecting interspecific gene exchange, population viability, species interactions and a series of ecological processes[9-11], which is a main cause of biodiversity decline and species endangerment and extinction[12-13]. Therefore, habitat fragmentation is very detrimental to the population development of *E. sanguineus*.

Table 3 The main components of associated and competitive species

Community	Trees	Shrubs	Herbs	Ferns
1	*Abies georgei Orr var. smithii*, *Pinus densata*, *Quercus aquifolioides*	*Ribes orientale*, *Spiraea bella*, *Rosa sikangensis*	*Fragaria nubicola*	*Dryopteris nyingchiensis*, *Drynaria delavayi*
2	*Quercus aquifolioides*, *Hippophae rhamnoides*	*Rhamnus virgata*, *Piptanthus concolor*, *Cotoneaster microphyllus*	*Duchesnea indica*	*Drynaria delavayi*
3	*Cupressus gigantea*, *Quercus aquifolioides*, *Hippophae rhamnoides*	*Piptanthus concolor*, *Hippophae rhamnoides*, *Caragana sinica*	*Pedicularis davidii*, *Hemiphragma heterophyllum*	*Drynaria delavayi*
4	None	*Sorbus rehderiana*, *Berberis amurensis*, *Caragana sinica*	None	*Drynaria delavayi*

5.2 Analysis of population structure

5.2.1 Analysis of plant population characteristics. By comparing the data of each sub-item in Table 4 and Fig. 1, it was found that the population density had a positive correlation with DBH, crown width, leaf length and branch length, but a negative correlation with tree height. The growth of plant population showed a horizontal development trend. It was found that the population density of *E. sanguineus* was very small, showing broken point distribution, and the population growth was slow, suggesting that its growth was a long-term accumulation process. The adult plants dominate the population and there are very few young plants, which makes the basic data about the population of *E. sanguineus* at a relatively high level. In fact, it reflected that the population of *E. sanguineus* was small, the spatial structure presented the random distribution, and the age structure presented the aging trend, so that its population structure was not complete and had no continuity.

Table 4 The basic characteristics of *Euonymus sanguineus* in different communities

Community	Density plant/ha	Average DBH // cm	Average tree height // m	Average crown diameter // m	Average leaf length // cm	Average current branch length // m	Average biennial branch length // m
1	4	17.9	4.74	3.42 × 3.16	5.24	0.42	0.51
2	3	15.6	4.46	3.84 × 3.61	5.06	0.34	0.47
3	6	19.4	4.37	4.33 × 4.01	6.88	0.49	0.59
4	8	20.2	3.89	4.68 × 4.26	7.67	0.53	0.62

5.2.2 Analysis of seedling survival. As shown in Table 5, it was found that there were current seedlings in and out of the crown diameter of plant, but there were no plants of more than two years in the crown diameter range, and there were few plants of more than two years in the area outside the crown diameter range. The number of young seedlings and adult plants were counted in different radius with the plant as the center from four sample plots. The number of seedlings in all sample plots decreased sharply with the increase of radius, and the number of plants over two years old was very small, and increased first and then decreased sharply with the increase of radius. The seeds of the four communities of *E. sanguineus* germinated and died one after another, and the number of seedlings more than two years old was also very small, and the survival amount of the seedlings was very small. The investigation also found that the surviving seedlings were in the area with the radius of more than 5 m, that is, it was beyond the crown coverage range (Table 6). The above two tables showed that the mobility of seeds was very weak, and they belonged to the indifferent species; the under-forest regeneration ability was poor, the survival rate of seedlings was very low, and the population was difficult to reproduce and develop.

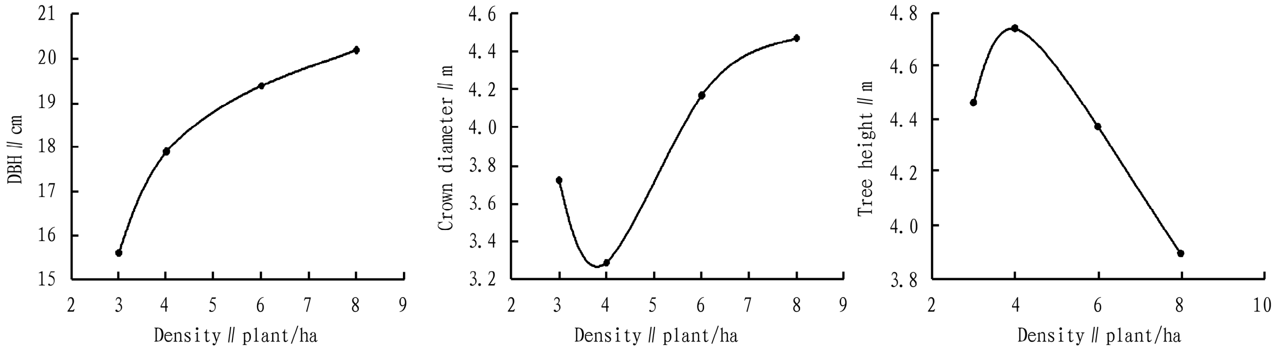

Fig. 1 The correlation between population density and DBH, crown diameter or tree height

Table 5 Survival rate of seedlings in different areas under canopy

Community	Age of seedlings	Item	Radius range // m			
			0 – 5	5 – 10	10 – 15	5 – 20
1	Current-year	Number of seedlings	44	35	23	12
	Two years or more		4	4	4	2
2	Current-year		32	26	18	9
	Two years or more		2	2	2	2
3	Current-year		70	62	51	34
	Two years or more		11	11	9	9
4	Current-year		95	87	70	51
	Two years or more		19	18	15	13

Table 6 Survival rate of seedlings in different periods

Community	Age of seedlings	Item	Number of days						
			0	20	40	60	80	100	120
1	Current-year	Number of seedlings	44	35	23	12	4	0	0
	Two years or more		4	4	4	2	2	2	1
2	Current-year		32	26	18	9	3	0	0
	Two years or more		2	2	2	2	2	2	2
3	Current-year		70	62	51	34	21	3	0
	Two years or more		11	11	9	9	9	6	3
4	Current-year		95	87	70	51	32	11	1
	Two years or more		19	18	15	13	13	8	6

5.2.3 Seed-setting ability. Seed is an important link in the life cycle of plants, and is the beginning of new life of young plants, related to the future fate of the population. Much attention has been paid to the ecological effects of seed size and quality on the successful settlement and survival of seedlings[14]. When the population of almost all species decreases, the most direct consequence is the increase of inbreeding coefficient and the subsequent inbreeding decline, and the decrease of population fitness[15–17]. The decrease of plant population fitness is reflected in a series of links in plant life cycle. Seed germination is a key link in plant life cycle, which often determines whether the population renewal is successful or not. The study on the germination of endangered plant seeds is helpful to exploring the mechanism of endangered plant seeds and to designing reasonable protective measures for endangered plant seeds[18].

As can be seen from Fig. 2, the average flowering and seed-setting rate of population in sample plot 4 was the highest among the 4 populations, while the number of seedless fruits was the lowest, which indicated that the seed-setting ability was relatively high. The seed-setting rate of all the *E. sanguineus* populations was very low, the highest seed-setting rate was only 43.0%, the lowest was only 38.6%, and the percentage of seedless fruits was more than 50%, showing a very low seed-setting ability. The statistics of seeds of 100 fruits showed that the percentage of full seeds was also small, the majority of seeds were damaged by insect pests, or rotten seeds and empty seeds, which greatly reduced the base of seed germination (Table 7). On the basis of the above two tables, it was found that the seed bearing ability of *E. sanguineus* was low, the pest was serious, and the seed fullness rate was very low, which made the seed germination probability greatly reduced, and it was adverse to the development of the population.

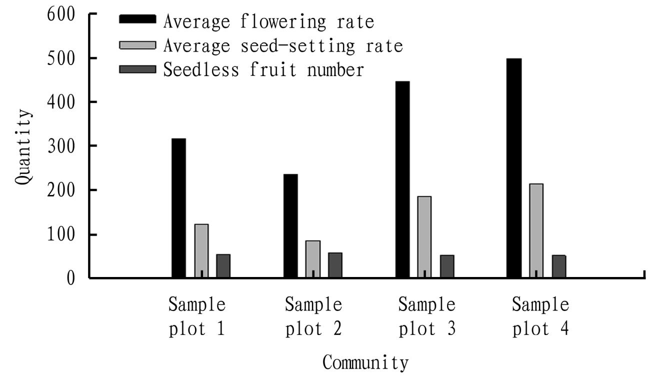

Fig. 2 Analysis of seed-setting ability of *Euonymus sanguineus* population in different communities

Table 7 Some basic characteristics of seeds in different communities

Community	Seed yield (per 100 fruits)	Number of empty seeds (abortion rate//%)	Number of rotted seeds (disease rate//%)	Number of pests (pest rate//%)	Number of full seeds (fullness rate//%)
1	180	58(32.2)	46(25.6)	49(27.2)	27(15.0)
2	172	61(35.5)	44(25.6)	27(15.7)	40(23.2)
3	192	53(27.6)	48(25.0)	30(15.6)	61(31.8)
4	196	52(26.5)	35(17.9)	29(14.8)	80(40.8)

5.3 Experiment on the key factors in seedling lethality According to the phenomenon of low survival rate of seedlings found in the investigation, it was suggested that the light, soil and litter might be the key factors of death, except the bad habitat conditions such as large temperature difference, low precipitation and human and animal disturbance. Therefore, the key factors of seedling lethality were studied.

5.3.1 Light. Light is an important environmental factor affecting seed germination and seedling growth and survival[19]. 50 seedlings of the same growth trend were taken from four investigation sites and planted in the original soil, and were treated with four kinds of light intensity. four groups of 10 plants, a total of 50 seedlings, were taken for a light intensity treatment. The seedlings of four groups were observed for three months. Table 8 showed that the seedlings of *E. sanguineus* grew well under 1/3 – 1/2 light intensity, followed by the growth status under full light intensity, and grew slowly under 1/5 – 1/4 light intensity, but no seedlings died. The measurement of light intensity under adult plants in four investigation sites showed that the light intensity was in the range of 3/10 – 4/5, but the seedlings died a lot. Because of the sharp contrast between the two, the light was not the key factor of seedling death.

Table 8 Growth status of seedlings under different light intensity

Community	Item	Light intensity				
		1/5 light	1/4 light	1/3 light	1/2 light	Full light
1	Growth status	+	+ +	+ + +	+ + + + +	+ + + +
2		+	+ +	+ + +	+ + + + +	+ + + +
3		+	+ +	+ + + + +	+ + + +	+ + +
4		+	+ +	+ + + + +	+ + + +	+ + +

Note: + slow growth; + + relatively slow growth; + + + average growth; + + + + relatively good growth; + + + + + good growth.

5.3.2 Soil. Soil acidity and alkalinity affect the chemical reaction, microbial activity and the availability of nutrients in the soil, thus affecting the physical and chemical properties of the soil and the growth of crops. The pH range of soil adapted to growth by different plants is different[20]. The soil used for "seedling growth under different light intensity" was native soil, and no seedlings died. Soil acidity and alkalinity testing results showed that the pH of the soil was in the range of 7.2 – 9.4, it was alkaline, and the seedlings were later planted in the acid soil. Table 9 showed that the seedlings of *E. sanguineus* could also grow in acid soil, and grew well in weak acid soil, but no seedlings died. Taken together, soil was not a key factor in seedling death.

Table 9 Growth status of seedlings in different acidic soils

Item	pH				
	5.4	5.8	6.2	6.6	6.8
Growth status	+ +	+ + +	+ + + +	+ + + + +	+ + + + +

Note: + slow growth; + + relatively slow growth; + + + average growth; + + + + relatively good growth; + + + + + good growth.

5.3.3 Allelopathy experiment on leaf litter. In order to verify whether leaf litter is the key factor of seedling death, a leaf allelopathy experiment was designed. Allelopathy refers to the interaction of biochemical substances among plants, and allelopathy has autotoxicity function. Autotoxicity among the same species is a mechanism for avoiding intraspecific competition derived from plant's long-term adaptation to the environment and evolutionary selection[21].

The collected leaves were washed, naturally dried, ground and sifted, and accurately weighed to be 1 g (dry weight), and treated with 50 mL of distilled water for 30 min of ultrasonic processing. After 48 h of extraction, it was centrifuged to obtain the supernatant, namely the original solution of extract. After it was mixed with distilled water, it was divided into four concentration gradients: 100%, 75%, 50%, 25%, with clear water as the control. The seedlings were cultured in solution and their condition was observed. As can be seen from Table 10, the seedlings died when they were put into the extract. The survival time of the seedlings increased with the decrease of concentration, while the seedlings of the control group were normal. Thus, it was found that the substances in the leaves caused the death of the seedlings. The results indicated that the leaves of *E. sanguineus* had autotoxic effect, which matched with the fact the seedlings of *E. sanguineus* could not survive under the plant. There were scarce under-forest and surrounding shrubs and herbs, and the simple structure also confirmed that the leaf of the *E. sanguineus* had autotoxicity.

Table 10 Experimental results of allelopathy

Concentration // %	Culture time // d								
	1	2	3	4	5	6	7	8	9
100	Leaf wilting	Branch and leaf withered	Died						
75	Leaf wilting	Stem initially lodging	Stem completely lodging	Branch and leaf withered	Died				
50	Normal	Leaf wilting	Leaf wilting completely	Stem partially lodging	Stem completely lodging	Branch and leaf withered	Died		
25	Normal	Normal	Normal	Leaf wilting	Leaf wilting completely	Stem partially lodging	Stem completely lodging	Branch and leaf withered	Died
Clear water	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

6 Conclusions and discussions

Taken together, it is known that: (i) The climatic and environmental conditions in the habitat of *E. sanguineus* were very bad, and the habitat was fragmented and spaced. (ii) The population development was influenced by competitive and associated species. Appropriate shrub encirclement reduced the disturbance of human and livestock and provided good growth space and resources, so that they could grow better. The protective layer established for the population was disturbed and destroyed by human and livestock. It was found that the natural habitat of *E. sanguineus* had been fragmented, and the growth of individuals, seed germination and seedling survival in the population had been greatly destroyed. (iii) The population was small, the density was small, and there were many adult plants but few young plants; the seed-setting ability was low, seed quantity of fruit was small, and disease and pest were serious; the seed movement ability was weak, germination probability was low, there were very few seedlings, and the population structure was in serious unbalance. (iv) The seed germination rate was greatly reduced by the autotoxicity of the substance in the leaves, and the seedlings died out under the autotoxicity. The autotoxicity of the litter was the key factor for the seedlings to die.

According to the investigation and statistics of habitat condition, population structure, seed-setting ability, seedling survival and the experiment of the key factors of seedling death, it was found that the main reasons for the population decline were as follows: (i) The *E. sanguineus* population was too small, and the survival area was too small, which seriously affected the interspecific mating and seed development and regeneration, and greatly reduced the genetic diversity within the population. (ii) The dry and hot river valley climate and human and animal activities in the investigation area inhibited the seed germination and seedling growth of *E. sanguineus*, resulting in very low survival rate of seedlings and hindering the population expansion of *E. sanguineus*. (iii) The distribution area is located in the Yarlung Tsangpo river valley where the natural habitat fragmentation has affected the interspecific gene exchange, leading to the low seed-setting ability and germination rate of *E. sanguineus*, which is the direct cause of its population decline. (iv) The litterfall of *E. sanguineus* had autotoxicity, which inhibited the growth of seedlings under the forest, caused slow natural regeneration of the population, and then caused the population of *E. sanguineus* to be unable to spread and develop.

The flower, fruit and leaf of *E. sanguineus* are graceful in shape, elegant, bright and changeable in color, which has great application value in garden. The potential of medicine, chemical industry, energy, environmental protection and other values of the plants of *Euonymus L.* also bring power to the research and development of *E. sanguineus*. Therefore, the investigation and study of its habitat adaptability and the reason of population decline can provide a theoretical basis for the future rational collocation of habitat conditions and species to make its landscape application and environmental protection better, and provide a theoretical basis for its species protection, breeding technology, large-scale introduction and cultivation, as well as the study of its exploitation and utilization.

In its follow-up work, attention should be paid to the following aspects: (i) It is necessary to increase the protection and management of *E. sanguineus* population, isolate, protect and improve its habitat, and reduce the habitat fragmentation caused by human and animal disturbance. (ii) The allelopathic autotoxin and its effect on seed germination and seedling growth should be further studied. (iii) The best method of seed germination and cuttage should be selected to protect the species of *E. sanguineus* and make it multiply in large area, then make it come back to its original habitat, and make it develop normally, so as to expand the population scale. (iv) The value potential of *E. sanguineus* should be studied and exploited to maximize its potential.

References

[1] ZHANG QX. Chinese ornamental plant germplasm resources · Tibet [M]. Beijing: China Forestry Publishing House, 2013. (in Chinese).

[2] ZHU H, YAN LH, WANG ZM, *et al.* Research progress on chemical constituents and pharmacological activities of medicinal plants of the genus *Euonymus* L. [J]. Chinese Pharmaceutical Journal, 2013, 48(4): 241-247. (in Chinese).

[3] KONG XQ, WU WJ. Isolation and bioactivities of the insecticidal components from the root of *Euonymus verrucosides*[J]. Acta Botanica Boreali-Occidentalia Sinica, 2000, 20(2):299-302. (in Chinese).

[4] HUANG JH, LIU GQ, BAI HJ, *et al.* Studies on insecticidal activities of 13 species of Celastraceae[J]. Acta Botanica Boreali-Occidentalia Sinica, 2004, 24(4): 688-692. (in Chinese).

[5] ZHANG QD, WANG MA, JI ZQ, *et al.* Sesquiterpene pyridine alkaloids from *Euonymus japonicus* and their insecticidal activity[J]. Acta Botanica Boreali-Occidentalia Sinica, 2007, 27(5):000983-988. (in Chinese).

[6] HE YH, DING ZL. Analysis on the oil of *Euonymus* L. seeds in Shaanxi [J]. Journal of Anhui Agricultural University, 1993, 20 (3): 200-203. (in Chinese).

[7] YE XF. Chemical composition analysis and evaluation screening of Chinese non-food biodiesel energy plants[D]. Guangzhou: Zhongkai University of Agriculture and Engineering, 2014. (in Chinese).

[8] XING Z, ZHANG QX, CI R. A preliminary survey on the habitat of *Paeonia ludlowii* in Tibet[J]. Jiangsu Agricultural Sciences, 2007, 35(4): 250-253. (in Chinese).

[9] ZHANG YX, MA KM, NIU SK. Metapopulation dynamic models: A novel approach to fragmented landscape dynamics[J]. Acta Ecologica Sinica, 2003, 23 (9): 1877-1890. (in Chinese).

[10] LI ZG, WANG YL, ZHANG XF, *et al.* Spatial-temporal dynamics of landscape fragmentation in North Shaanxi Loess Plateau [J]. Chinese Journal of Applied Ecology, 2005, 16(11):2066-2070. (in Chinese).

[11] YANG F, HE DH. Effects of habitat fragmentation on biodiversity[J]. Ecological Science, 2006, 25 (6): 564-567. (in Chinese).

[12] WU ZJ, LI YM. Effects of habitat fragmentation on survival of animal populations[J]. Acta Ecologica Sinica, 2003, 23(11): 2424-2435. (in Chinese).

[13] LIU JF, XIAO WF, JIANG ZP, *et al.* A study on the influence of landscape fragmentation on biodiversity[J]. Forest Research, 2005, 18(2): 222-226. (in Chinese).

[14] PENG SJ, HUANG ZL, PENG SL, *et al.* Factors influencing mortality of seed and seedling in plant nature regeneration process[J]. Guihaia, 2004, 24(2): 113-121. (in Chinese).

[15] CRNOKRAK P, ROFF DA. Inbreeding depression in the wild[J]. Heredity, 1999, 83(3):260-270.

[16] KELLER LF, WALLER DM. Inbreeding effects in wild populations[J]. Trends in Ecology & Evolution, 2002, 17(5):230-241.

[17] REED D, FRANKHAM R. The correlation between population fitness and genetic diversity [J]. Conservation Biology, 2003, 17 (1): 230-237.

[18] HU SJ, HE P, WANG RB, *et al.* Seed germination characters of populations of the endangered plant *Euonymus chloranthoides* [J]. Scientia Silvae Sinicae, 2007, 43(5):42-47. (in Chinese).

[19] YAN XF, WANG JL, ZHOU LB. Effects of light intensity on *Quercus liaotungensis* seed germination and seedling growth[J]. Chinese Journal of Applied Ecology, 2011, 22(7): 1682-1688. (in Chinese).

[20] LI H. Advanced plant nutrition[M]. Beijing: Science Press, 2003. (in Chinese).

[21] LI XY, LIANG ZY, BIAN XJ, *et al.* The allelopathic effect and application of trees[J/OL]. Molecular Plant Breeding, 2017, 15(4): 1-10. http://kns.cnki.net/kcms/detail/46.1068.S.20170412.1550.002.html. (in Chinese).