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Interspecific Correlation between *Cephalcia kunyushanica* and Host Plant in Natural Forest in Kunyushan Mountains, China

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Abstract. In order to determine the propensity of *Cephalcia kunyushanica* (Hymenoptera: Pamphiliidae) to different forest types (host stand and host-non-host mixture stand) and the interaction between host amount and larvae density of *Cephalcia kunyushanica*. We investigated the mean insect density and stand types in 50 permanent plots in the Kunyushan Mountains, China. The results showed that seven kinds of pine species were determined as the host of *Cephalcia kunyushanica* in Kunyushan Mountains. Among the seven species, difference in larvae density was not significant ($p > 0.05$). Likewise, non-significant difference ($p > 0.05$) was also found in the mean density of the insects between two kinds of stand types (pure host stand and mixed stand). Strong negative linear relationship between mean insect density and host amounts were found in pure host plots in the year 2013 and 2014. However, non-significant ($p > 0.05$) correlation was found in host-non-host mixture plots. In addition, in 39 plots investigated, the Shannon-Wiener index (H') did not change significantly ($p > 0.05$) in 2008 and 2013, indicating that the resource dilution effect fit the *Cephalcia kunyushanica*-host model in the Kunyushan Mountains. The current manuscript reports the interaction between the specialist phytophagous insects and the hosts is fit for the resource dilution hypothesis. The result will improve the understanding and operation on the insect-host relationship, as well as the hypothesis could be helpful for us in ecological controlling forest pests.

INTRODUCTION

A deep insight interaction between insects and their hosts is beneficial for the biological prevention and control in forest management. Kunyushan web-spinning sawfly, *Cephalcia kunyushanica* (Hymenoptera: Pamphiliidae), was found firstly in 1983 and identified as a new species in 1990 [1]. It exists uniquely in Kunyushan Mountains so far and has been an integral part of the Japanese red pine (JRP) forest ecosystem in Kunyushan Mountains, China.

Although it has existed for more than 20 years since the last severe outbreak of the sawfly [2], the reason of the outbreak and knowledge on how to prevent and control the *Cephalcia kunyushanica* density are still inadequate.

The relationship and interaction among species in the same environment have long been a focus in community ecology study, which is called “interspecific affinity”. The “interspecific affinity” includes “interspecific correlation” and “interspecific association”. The interspecific correlation describes the quantitative relationships between different species and the interspecific association depicts the spatial patterns of different species coexisting in a particular region by using the binary qualitative analysis model [3-5]. Both interspecific correlation and interspecific association are based on the research of biological diversity, especially plant diversity, which plays an important role in insect diversity [6].

Many mechanisms including the diversity-stability hypothesis [7-8] (Goodman, 1975; McCann, 2000) and the associational resistance hypothesis [9] have been used to explain how phytophagous insects were affected by plants diversity [10]. The diversity-stability hypothesis states that the stability has a positive correlation with the diversity of a community, and the populations of both forest herbivores and their enemies should present a lower degree of temporal variation in more diverse stands [11]. Associational resistance is based on the interactions among a plant, its

phytophagous insects, and the surrounding plant species [9]. Associational resistance is derived from the resource concentration hypothesis and the enemies hypothesis [12].

Many studies support the resource concentration hypothesis, indicating that the herbivores were positively related with the abundant host plants. The resource concentration hypothesis also can explain the relationship among patch area, population density and the scaling of migration rates [13]. At the same time, researchers like Yamamura [14] have found evidence to support resource dilution in the area of natural and semi-natural communities.

Most of the studies about the resource concentration hypothesis have focused on herbivores in agricultural ecosystem like orchards and cultivated fields, which are characterized by monocultures or low-diversity mixtures [15]. However, natural and semi-natural communities are more likely to support resource dilution hypothesis. Englund and Hambäck [13] even found both the phenomenon of resource concentration and resource dilution existed at the different stage of the host plants. These studies have found that stand types, host density, and even biodiversity can influence the density, or loads, of specialist insect herbivores [16].

However, the complex set of relationships between specialist insect herbivores and their hosts in Kunyushan Mountains remains unclear in presence of abovementioned hypotheses. The association between *Cephalcia kunyushanica* and 195 neighboring shrub or herbage plants had been studied based on the sample-circle method and the 2×2 contingency table in our previous study [2]. The results indicated that there was a significant positive correlation of overall association existing among *Cephalcia kunyushanica* and 195 neighboring shrub or herbage plants. Like shrub and herbage plants, arborous are also components of forest community structure, the quantitative relationship between *Cephalcia kunyushanica* and their host plants was needed. In this study, we sought to parse the inter-specific affinity impact to the population of the leaf-eater insects for the Kunyushan natural *Pinus densiflora* pure and mixed forest ecosystem. *Cephalcia kunyushanica*-host system was so stable that the (*Cephalcia kunyushanica*) herbivore didn't emerge largely for more than 20 years [2].

The purpose of this paper was to (1) reveal the quantitative relationship between *Cephalcia kunyushanica* and their host plants by interspecific correlation analysis, (2) examine which hypothesis (resource concentration hypothesis, resource dilution hypothesis or both) fit *Cephalcia kunyushanica*-host system, (3) provide the scheme of stand type for ecological controlling the larval density in Kunyushan Mountains, China.

MATERIALS AND METHODS

Study Species

Cephalcia kunyushanica (Hymenoptera: Pamphilidae), one species of specialist phytophagous insects [1, 17-19], exists only in the Kunyushan Mountains and feeds mainly on Japanese red pine (*Pinus densiflora*), occasionally on China armand pine (*P. armandii*), Korean pine (*P. koraiensis*), and lodgepole pine (*P. thunbergii*). Its population lives in pine trees of all ages and outbreaks irregularly and severely in Kunyushan Mountains. The adults appear in May or June every year, and lay eggs in groups on pine needles. Larvae spin silk nest and generally two to four live in one nest after hatching [1]. At the base of the nest or in a feces-covered silk tube, they cut and eat needles, basically never actively disperse in the larval stage from June to August. Then they drill a 5-12 cm depth hole into the tree and prepare to overwinter. Pupation takes place in the next April and about one month later the adults emerge [20].

Study Area and Plot Design

The Kunyushan Mountains (121°41'34"-121°48'04" E, 37°11'50"-37°17'22" N) lies in the Jiaodong peninsula of Shandong province, China, covering an area of 15416.5 km². The climate is moderate with a mean temperature of 11.8 °C, a frost-free period of 200-220 days, and an annual precipitation of 800-1000 mm. The main indigenous broadleaf species are sapphireberry sweetleaf (*Symplocos paniculata*), roundleaf buckthorn (*Rhamnus parvifolia*), holm oak (*Quercus acutissima*), mountain ash (*Sorbus alnifolia*), and thorny elaeagnus fruit (*Elaeagnus pungens*). The main indigenous conifer species, Japanese red pine, naturally distributes from the piedmont to the highest area of the mountains at 800 m above sea level. Other conifer species including the China armand pine (*P. armandii*), lodgepole pine (*P. thunbergii*), jack pine (*P. banksiana*), Chinese fir (*Cunninghamia lanceolata*), pitch pine (*P. resinosa*), Korean pine (*P. koraiensis*), and larch (*Larix gmelinii*) were introduced in the 1960s and planted randomly forming unique forest stands ranging in size from 2.5 ha to 25 ha. Nowadays, the majority of pure species plots have been converted into mixed ones with secondary *P. koraiensis*, aged between 20 and 45 years, due to pest outbreaks in the 1970s [20]. In 2006 and 2012, fifty 30×30 m² permanent plots were established, and the species accumulation curve

proved that 50 plots can comprehensively reflect the types of major forest communities, stand structure and species diversity in Kunyushan Mountains.

The precise longitude and latitude of the 50 plots were measured by GPS at the center of each plot. Eight cement columns were used to create permanent markers. Four of the columns were set at intersection points of two borders and the other four were fixed at the middle along each plot border. Site features for each plot, like angle of gradient, elevation, and slope aspect, were then recorded.

Seasonal Mature Larvae Density of *Cephalcia kunyushanica*

Web spinning and nesting in branches were used as evidence of infestation for *Cephalcia kunyushanica* larvae. Insect nests on them were observed with a binoculars and their numbers were counted tree by tree (the tree density in each plot see Table 2) in each plot every 3 days from July to August of each year during the study. The larval amount of *Cephalcia kunyushanica* per tree, i.e. the larval density, was calculated by trebling the nest numbers, for each nest usually contained three larvae in average.

From August, the larvae start to drop from hosts and burrow into the ground, living and mature larvae were collected in the plot and placed on some plates.

Statistical Analysis

The relationship between mean larval density standing for the sawfly occurrence and host density was detected by using Pearson's correlation analysis and two-tailed significance tests. The data was transformed by \ln_x , where x is the mean larval density or host density. According to the results of the plant species diversity investigation, the Shannon-Wiener index of arbor plants in each plot was calculated by the equation (1):

$$H' = -\sum P_i \ln P_i \quad (1)$$

where the P_i means the appearance frequency of species i in the plot.

Then, the mean of the Shannon-Wiener index for each plot for the period was compared between 2008 and 2013 by using Tukey's t test ($\alpha = 0.05$). The difference of the mass of larvae sampled from the different hosts was tested by a one-way ANOVA using Fisher's f -test ($p < 0.05$). The mean density of larvae from hosts in pure host stands was compared with the density of larvae from hosts in the host-non-host mixed stand.

RESULTS

Host Range of *Cephalcia kunyushanica*

In this study, the *Cephalcia kunyushanica* was found in 39 permanent plots accounting for 75% of the total plots sampled in the Kunyushan Mountains. There were seven species of pine trees parasited by this herbivore pest, including *Pinus densiflora*, *P. koraiensis*, *P. thunbergii*, *P. armandii*, *P. taeda*, *P. banksiana*, and *P. resinosa*. Among the seven species, *P. koraiensis* had the largest mean larval mass with 0.255 g/head, yet *P. taeda* and *P. resinosa*, both the smallest with 0.170 g/head. However, a single-factor nonhomogeneous analysis revealed no significant difference ($p > 0.05$) in the mass of larvae among the seven species (Table 1).

TABLE 1. Larval mass differences detected by ANOVA

Host species	Mean (g / head)	Std. error	F-value	P-value *
<i>Pinus densiflora</i>	0.209	0.106		
<i>P. thunbergii</i>	0.205	0.093		
<i>P. koraiensis</i>	0.255	0.096		
<i>P. banksiana</i>	0.179	0.095	1.888	0.087
<i>P. taeda</i>	0.170	0.082		
<i>P. armandii</i>	0.208	0.104		
<i>P. resinosa</i>	0.170	0.065		

***Note:** $P < 0.05$ indicated the difference was significant.

Occurrence of *Cephalcia kunyushanica*

A Pearson's correlation analysis showed that there was no significant relationship ($p > 0.05$) between sawfly larval density and the number of hosts in host-non-host mixed plots in 2013 and 2014 ($R^2_{2013}=0.00$, $R^2_{2014}=0.03$). However, in pure host plots, with increasing host-plant density, the larval density decreased linearly and significantly ($p < 0.05$) ($R^2_{2013} = 0.54$, $|t_{2013}| > |t_{(0.05,17)}|$; $R^2_{2014} = 0.29$, $|t_{2014}| > |t_{(0.05,17)}|$) (Figures 1-4, Table 2).

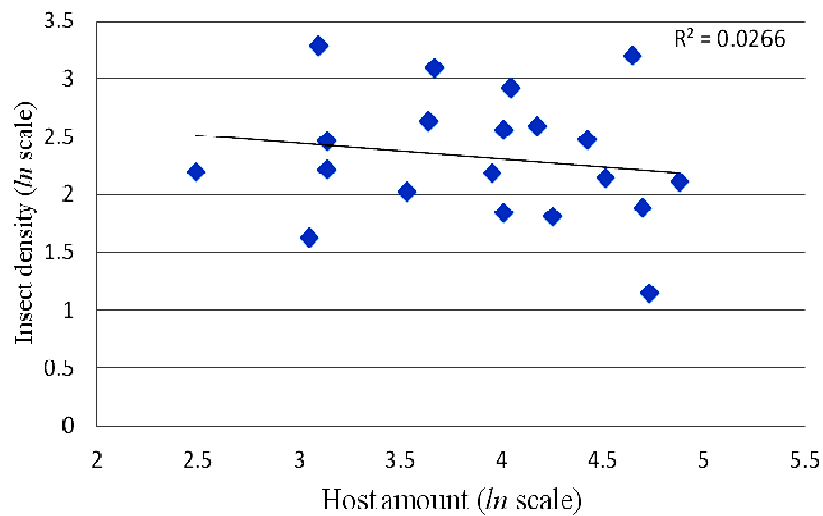


FIGURE 1. The relationship between host amount and insect density in pure host plots in 2013. **Note:** The value of host amount and insect density was switched by logarithms, and the same below.

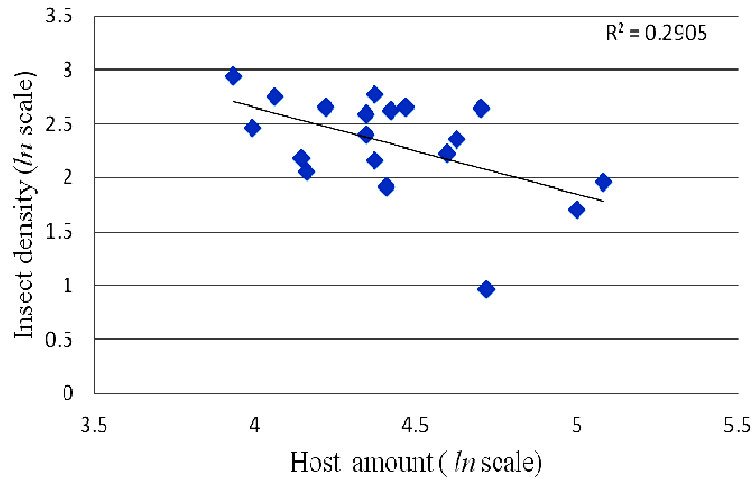


FIGURE 2. The relationship between host amount and insect density in pure host plots in 2014

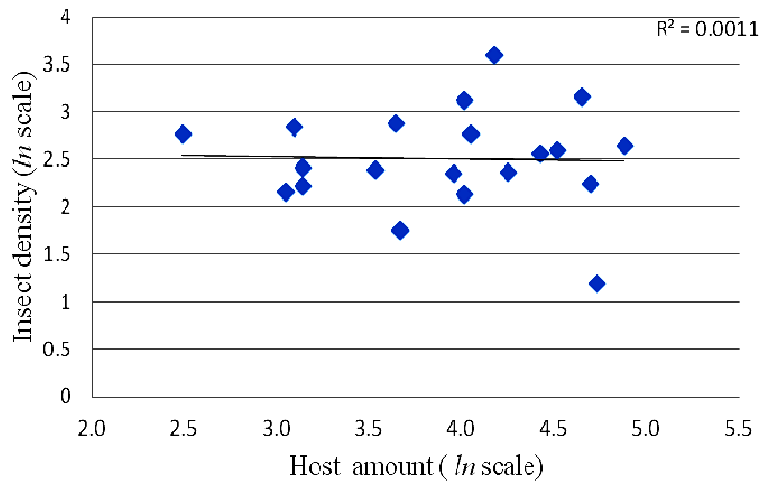


FIGURE 3. The relationship between host amount and insect density in mixture plots in 2013

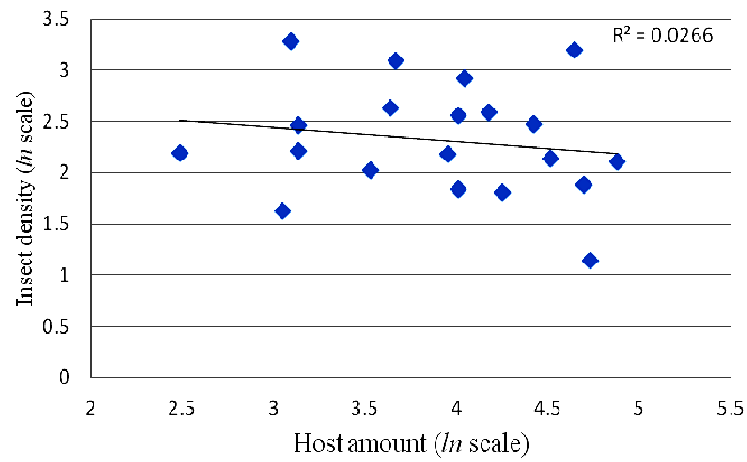


FIGURE 4. The relationship between host amount and insect density in mixture plots in 2014

TABLE 2. The Shannon-Wiener index and detailed information about the 39 plots and mean larval density in hosts

Plot No.	Stand type	Amount of trees	Amount of hosts	The Shannon-wiener index		Mean larval density (head / tree)	
				2008	2013	2013	2014
1	<i>Pinus densiflora</i>	62	51	0.2469	0.1279	27.6±2.2	19.1±1.4
2	<i>P. taeda</i>	74	63	0.6508	0.6548	17.6±0.8	8.9±0.4
3	<i>P. densiflora</i>	80	64	0.6439	0.6421	22.2±0.9	7.8±0.9
4	<i>P. densiflora</i>	61	58	0.4034	0.3792	27.4±1.6	15.6±1.5
5	<i>P. densiflora</i> × <i>P. thunbergii</i>	109	87	1.1802	0.9313	13.2±1.1	14.2±1.0
6	<i>P. densiflora</i>	91	77	0.7244	0.7496	14.8±0.6	11.0±1.2
7	<i>P. densiflora</i>	117	54	1.837	1.8152	15.6±1.0	11.7±0.9
9	<i>P. densiflora</i> × <i>P. thunbergii</i>	105	102	0.6297	0.6365	16.5±1.3	10.6±1.1
10	<i>P. densiflora</i> × <i>P. thunbergii</i>	117	77	1.2258	1.2085	16.9±0.8	13.4±1.2
11	<i>P. densiflora</i> × <i>P. thunbergii</i>	146	110	0.7002	0.6765	8.6±0.4	14.1±1.0
12	<i>P. densiflora</i> × <i>P.thunbergii</i> × <i>non</i>	137	91	1.373	1.3286	13.4±1.1	8.6±0.4
13	<i>P.thunbergii</i>	87	79	0.3326	0.3279	11.4±0.7	8.7±0.3
14	<i>P. densiflora</i> × <i>P. thunbergii</i> × <i>non</i>	184	131	1.1223	1.1119	14.0±1.1	8.3±0.6
16	<i>P. densiflora</i> × <i>non</i>	117	55	0.7633	0.7387	8.4±0.7	6.3±0.4
17	<i>P. densiflora</i> × <i>P. thunbergii</i>	90	83	0.4846	0.4902	13.8±1.1	13.8±1.3
18	<i>P. densiflora</i> × <i>P. thunbergii</i> × <i>non</i>	137	104	0.7749	0.9005	23.5±2.1	24.6±2.4
19	<i>P. densiflora</i> × <i>P. thunbergii</i> × <i>non</i>	157	57	0.98	1.2227	15.9±1.3	18.7±1.8
21	<i>P. densiflora</i> × <i>P. thunbergii</i> × <i>P. resinosa</i>	181	161	1.0716	1.0919	5.8±0.4	7.1±0.4
22	<i>P. densiflora</i> × <i>P. banksiana</i>	118	99	1.3633	1.3384	13.4±1.3	9.3±0.9
23	<i>P. densiflora</i> × <i>P. thunbergii</i>	99	79	1.3267	1.3545	20.4±1.7	16.0±0.8
24	<i>P. densiflora</i> × <i>P. thunbergii</i> × <i>P. taeda</i>	86	82	0.7697	0.7749	15.3±1.0	6.8±0.5
25	<i>P. densiflora</i> × <i>P. armandii</i> × <i>non</i>	127	65	1.5241	1.45	36.5±1.9	13.3±0.7
26	<i>P.thunbergii</i> × <i>non</i>	286	109	1.1411	1.1208	9.4±0.4	6.6±0.4
27	<i>P. densiflora</i> × <i>non</i>	103	55	1.2378	1.3823	22.8±1.8	12.9±1.1
32	<i>P. densiflora</i> × <i>P.thunbergii</i> × <i>non</i>	72	52	1.1928	1.1928	10.5±0.5	8.9±0.7
33	<i>P.densiflora</i> × <i>P.thunbergii</i>	119	112	0.8381	0.8405	1.0±0.1	2.6±0.2
35	<i>P. densiflora</i> × <i>non</i>	83	70	0.5859	0.4163	10.6±0.9	6.1±0.3
36	<i>P. densiflora</i>	67	68	0.0776	0	16.3±1.4	14.3±1.0
37	<i>P. densiflora</i> × <i>non</i>	201	21	0.6508	0.6245	8.6±0.5	5.1±0.3
38	<i>P. densiflora</i> × <i>non</i>	147	34	1.8005	1.254	10.9±0.9	7.6±0.7
39	<i>P. densiflora</i> × <i>non</i>	157	83	0.6869	0.7569	13.0±1.2	11.9±1.0
40	<i>P. densiflora</i> × <i>non</i>	101	38	1.1034	1.133	17.8±0.9	14.0±1.1
41	<i>P. thunbergii</i>	149	148	0.1638	0.1638	1.1±0.1	5.5±0.3
42	<i>P. densiflora</i> × <i>non</i>	162	23	0.7892	0.7606	11.1±0.9	9.2±0.6
43	<i>P. densiflora</i> × <i>non</i>	108	22	1.5019	1.5102	17.2±1.4	26.9±1.9
44	<i>P. densiflora</i> × <i>non</i>	191	113	0.8087	0.8102	3.3±0.2	3.2±0.1
45	<i>P. densiflora</i> × <i>non</i>	110	23	1.1951	1.1742	9.2±0.4	11.7±0.6
47	<i>P. densiflora</i> × <i>non</i>	82	12	1.9994	2.0036	16.0±1.0	9.0±0.4
49	<i>P. densiflora</i> × <i>non</i>	115	39	1.2736	1.2683	5.8±0.3	22.0±1.3
Mean				0.9532 ^a	0.9324 ^a	14.3±0.8 ^a	11.4±0.6 ^a

* **Note:** “non” in the table indicated non-host plants. The means with the same letter “a” were not significantly different by using Tukey’s t test ($\alpha=0.05$). The data of average larval density in 2013 and 2014 were indicated with mean value ± standard error.

The mean value of Shannon-Wiener index of the 39 plots in 2008 (0.9532) was nearly the same as in 2013 (0.9324), with a statistically non-significant difference (Table 2). It is suggested that this type of dominant defoliating insect species had no significant influence on the species-richness of the 39 plots, where different kinds of host pines were distributed.

DISCUSSION

The resource concentration hypothesis states that the abundance of insects (particularly specialist herbivores) per plant increases with the host density and plot size, in other words, insect individuals are more likely to find and remain in plots where host plants are concentrated [12]. If the hypothesis fit the insect-host relationship in our research, the average amount of defoliating insects would increase with the increased amount of host in the plots. However, our results showed no significant difference in the mean density of the insect between two kinds of stand types, pure host stand and host-non-host mixed stand, for a two-year investigation. It indicated, at least in this region, that this species of sawfly responded hardly to the stand type difference, and our results did not meet with the resource concentration hypothesis, which is the most influential hypothesis [5].

The dominant defoliating insect in Kunyushan Mountains, *Cephalcia kunyushanica*, had no obvious influence on the arbor species richness, which could be an evidence object to the resource concentration hypothesis.

Our further study evidence challenged the resource concentration hypothesis and showed that insect density decreased linearly with increasing host-plant density in fixed size plots (Figure 1). Although the correlation coefficients for 2013 and 2014 differed, which might due to the inter-annual fluctuation of weather conditions, the test was valid and the trend definitely showed a resource dilution effect. This hypothesis can be used in forest management, reducing the density of arbors by thinning, which can make the hosts give full play to isolate the phytophagous insect [21].

The aim of our analysis was to find an adjustment method on the insect-host mechanism, although the ecosystem here is so stable that no outbreak of this specialist insect herbivore occurred for over 20 years. Therefore, the potential mechanism could be helpful for us in controlling forest pests.

It is hard to know whether mass comparison is the best method for detecting bottom-up forces, but it is clear that measures beyond density are needed. Otway *et al.* [22] used their own index based on load to evaluate trends and to determine the resource dilution effect; however, the hosts in their research were all herbaceous plants, which are easily operational and the resulting data is easy to obtain. The method is not effective for arbor plants.

It is unclear why the insect-host system showed resource dilution effects. This is not any different from previous studies on this matter, which also do not provide explanations. It seemed that the total number of larvae in each plot provided a good balance, so that the larval density decreased linearly with increasing host-plant density. This suggests that the distribution patterns likely depended mainly on the biological characteristics of *Cephalcia kunyushanica*. Data from a test we carried out to determine the larval survival rate in the field revealed an extremely low result: 18 of 1069 (unpublished data obtained in 2013). The innate disadvantage of the viability of the specialist herbivores insect made it difficult for them to survive in nature, which may directly lead to their distribution patterns. So, we guess that in similar research, the biological characteristics of the key insects should be taken into account, and not be omitted.

Cephalcia kunyushanica, as a kind of endemic species, had an important effect in diversity of KunYushan Mountains ecosystem. The dynamic phenomenon observed from the specialist herbivores insect-host may add to forest pest management theory, study, practice, and application. Therefore, evaluating whether *Cephalcia kunyushanica* plays the role in that ecosystem timely and reasonably would be of use for their development and conservation.

Our research led to the question, “How can we evaluate the resource effect if the mass had significant differences among the samples from different hosts?” Our team did not address this question, but others might, because “Such effects have been found elsewhere and may be more widespread than thought previously” [22].

CONCLUSION

The *Cephalcia kunyushanica* was found in 39 permanent plots accounting for 75% of the total plots sampled in Kunyushan Mountains and there were seven species of pine trees invaded by this herbivore pest. Pearson’s correlation analysis indicated the resource dilution effect fit the *Cephalcia kunyushanica*-host model in Kunyushan Mountains.

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