

Effects of Roads on *Castanopsis carlesii* Seedlings and Their Leaf Herbivory in a Subtropical Forest in China

Authors: Dai, Xiao-Hua, Xu, Jia-Sheng, and Cai, Lu-Rong

Source: Journal of Insect Science, 14(17) : 1-13

Published By: Entomological Society of America

URL: <https://doi.org/10.1673/031.014.17>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.



Effects of roads on *Castanopsis carlesii* seedlings and their leaf herbivory in a subtropical forest in China

Xiao-Hua Dai^{1,2a*}, Jia-Sheng Xu^{1b}, Lu-Rong Cai^{1c}

¹School of Life and Environmental Sciences, GanNan Normal University, Ganzhou 341000, China

²National Navel-Orange Engineering Research Center, Ganzhou 341000, China

Abstract

The effects of a forest road on *Castanopsis carlesii* (Hemsley) Hayata (Fagales: Fagaceae) seedlings and their leaf herbivory were investigated in a subtropical forest at Jiulianshan National Nature Reserve, Jiangxi, China. A total of 1124 seedlings, 33949 leaves, 468 leaf mines, and 205 leaf galls were found. Generally, individual numbers, tree heights, and leaf numbers of *C. carlesii* seedlings became lower with increasing distances from the road. These results might indicate that old seedlings were fewer and survival rate of seedlings was lower in forest interiors. Leaf miners preferred the seedlings close to the forest road, while leaf galls preferred the seedlings about 2 m from the road. Species diversity of leaf miners was higher in the forest interior area, while species diversity of leaf galls was higher near the road. However, both leaf miners and leaf galls decreased in general from the road to the interior forest. There were interspecific differences in the effects of roads on leaf miner species and leaf galler species. The effects of the road on seedlings and insects could be explained by varying microhabitat conditions and different ecological strategies.

摘要

调查了中国江西九连山国家级自然保护区亚热带森林中森林小路对米槠幼苗及其食叶昆虫的影响。研究共发现 1124 棵幼苗, 33949 个叶片, 468 个潜道和 205 个虫瘿。总体而言, 随着与小路距离的增加, 米槠幼苗的个体数、树高和叶片数都随之减小, 说明在森林内部很少年龄较大的幼苗而且幼苗的存活率很低。潜叶昆虫偏嗜距离森林小路很近的幼苗, 但叶瘿昆虫却偏嗜距离小路 2 m 左右的幼苗。潜叶昆虫的物种多样性在森林内部更高, 而叶瘿昆虫的物种多样性在小路附近更高。但是, 从路域到植被内部, 潜叶昆虫和叶瘿昆虫总体上均呈减少趋势。路域对潜叶昆虫或造瘿昆虫的影响存在种间差异。幼苗和昆虫的这些路域效应可通过微生境的变化和生物的生态对策来解释。

Keywords: leaf miners, leaf galls, road ecology, species diversity

Correspondence: ^aecoinformatics@gmail.com, ^b419893498@qq.com, ^c1031345447@qq.com, *Corresponding author

Editor: Nadir Erbilgin was editor of this paper.

Received: 27 May 2012 **Accepted:** 8 December 2012 **Published:** 5 February 2014

Copyright: This is an open access paper. We use the Creative Commons Attribution 3.0 license that permits unrestricted use, provided that the paper is properly attributed.

ISSN: 1536-2442 | Vol. 14, Number 17

Cite this paper as:

Dai X-H, Xu J-S, Cai L-R. 2014. Effects of roads on *Castanopsis carlesii* seedlings and their leaf herbivory in a subtropical forest in China. *Journal of Insect Science* 14:17. Available online: <http://www.insectscience.org/14.17>

Introduction

Roads have large ecological effects on plants and insects. First, a road can change atmospheric and soil conditions. Microhabitat conditions such as pollutants, light, temperature, moisture, and nutrients vary between locations near roads vs. far from roads (Spencer and Port 1988; Spencer et al. 1988; Angold 1997; Avon et al. 2010).

With the above microhabitat variations at different distances from roads, plant characteristics will change accordingly. Generally, litter depth, canopy cover, canopy height, basal area, population density, total biomass, species diversity of native plants, shade-requiring plants, and geophytes are smaller at road edges (Watkins et al. 2003; Godefroid and Koedam 2004; Flory and Clay 2009; Avon et al. 2010), while understory cover, species diversity of exotic plants, graminoids, heliophilous plants, disturbance species, and therophytes are higher when adjacent to the roads (Watkins et al. 2003; Godefroid and Koedam 2004; Avon et al. 2010). Plant individuals have higher body size, biomass, dry weight, nitrogen, water potential, lead, sodium, manganese, leaf number, flower number, shoot length, and shoot diameter on road edges (Spencer and Port 1988; Spencer et al. 1988; Lightfoot and Whitford 1991; Martinez and Wool 2006), but individuals have lower resin and sulfate near the roadside (Lightfoot and Whitford 1991). Native plants germinate, survive, and grow better away from roads (Martel 1995; Comita and Goldsmith 2008), while exotic plants perform better near roads (Flory and Clay 2009). However, plants of different species or different ages may show different responses to road-distance effects (Angold 1997; Flory and Clay 2006; Avon et al. 2010).

Finally, along with effects of roads on atmosphere, soil, and plants, animals will distribute and behave differently at different distances from roads. For vertebrates and macroinvertebrates, abundance, diversity, density, pairing success, reproduction, survival, and path tortuosity become higher away from roads, while territory size, dispersal distance, mortality, and lead concentration become lower away from roads (Reijnen et al. 1995; Ortega and Capen 2002; Marsh and Beckman 2004). For insects, abundance, diversity, density, development time, reproduction, and fecundity become lower away from roadside regions (Muskett and Jones 1980; Spencer and Port 1988; Lightfoot and Whitford 1991; Koivula 2005; Martinez and Wool 2006; Dunn and Danoff-Burg 2007). However, animals of different species, different sexes, and different ages may respond differently to roads (Reijnen et al. 1995; Ortega and Capen 2002; Marsh and Beckman 2004; Martinez and Wool 2006). Distance effects of roads on plants, insects, and animals may vary with different scales and with different road types (Forman and Alexander 1998; Dunn and Danoff-Burg 2007; Comita and Goldsmith 2008; Avon et al. 2010).

Castanopsis carlesii (Hemsley) Hayata (Fagales: Fagaceae) is a dominant tree species in the subtropical evergreen broadleaf forest in South China. In natural habitats, seedling banks of *C. carlesii* are abundant in forest understory. Moreover, plenty of insect herbivory patterns, including leaf mines and leaf galls, are found on *C. carlesii* adults and seedlings (Wang et al. 2006). Mines and leaf galls are distinctive marks of endophagous organisms on host plants (Krassilov 2008). Most miners and gallers are monophagous or oligophagous, while structures of many mines and galls are species-specific or genus-specific (Dai et al. 2011). Since the types and numbers of leaf

mines and leaf galls can be easily identified, mines and galls are ideal for studies on road effects.

In this study, small-scale effects of a forest road on leaf herbivory of *Castanopsis carlesii* seedlings in a subtropical forest were quantified. The following two questions were addressed: 1) Were there different responses by plant seedlings, leaf miners, and leaf gallers to roads? 2) Were there interspecific differences in the effects of roads on leaf miner species leaf galler species?

Materials and Methods

Study site

Jiulianshan National Nature Reserve (24° 29' 18"–24° 38' 55" N, 114° 22' 50"–114° 31' 32" E) is located in the south of Jiangxi Province, China. It covers 134 km² with an altitude range from 280 to 1,434 m a.s.l. The climate is subtropical, and the annual precipitation is 2,156 mm. The mean monthly temperature ranges from 6.8°C (January) to 24.4°C (July). The dominant vegetation is subtropical evergreen broadleaf forest, low hill coniferous forest, bamboo forest, montane dwarf forest, and montane grassland (Liu et al. 2002). *C. carlesii* is one dominant tree species in the area.

Data collection

The seedlings of *C. carlesii* mainly occurred in forest edges. A mountain ridge was chosen because there were plenty of seedlings (Figure 1). Along a small forest road under the forest cover, multiple line transects were set perpendicular to the road. The distances between two transects was 5 m. A total of 19 transects on one side of the road and nine transects on the other side were sampled. All seedlings inside ± 0.5 m to each transect were investigated. Each transect was divided 1 m after 1 m from

the road so a series of 1 m² areas along one transect could be therefore obtained. Generally, the number of complete squares along one transect was fewer than six (Figure 1). Distance classes were set according to the midpoint of each square, that is, 0.5 m, 1.5 m, 2.5 m, etc. The last square for each transect wasn't used, since it was usually incomplete. The identification of leaf mines and leaf galls were based on mine or gall characteristics. In previous studies, some leaf-mining species had been reared and identified (Dai et al. 2013).

The number of seedling individuals, number of seedling leaves, accumulated tree height, number of mines per leaf, number of galls per leaf, number of each leaf mine type, and number of each leaf gall type were calculated for all seedlings in each complete square. Mine diversity and gall diversity were measured at each distance class by both Simpson's diversity index (*D*) and Shannon's diversity index (*H*).

Statistical analysis

Generalized additive models (GAM) were used to describe plant and herbivory parameters in relation to distance from the road. GAM approach was used because no *a priori* assumptions should be made about the relationship (Hastie and Tibshirani 1990; Rokaya et al. 2012). GAM fitting with a smoothing spline was performed with PAST version 2.15 (Hammer et al. 2001). All data points at the same distance class were collapsed to a single point by weighted averaging and calculation of a combined standard deviation. Since standard deviation was unspecified for *D* or *H*, it was set to the default 10% of the standard deviation of *D* or *H* in PAST program. Optimal smoothing was calculated by a cross-validation procedure. The optimal smoothing

factor of De Boor (2001) was thus obtained to fit a third-order polynomial spline.

Results

A total of 1,124 seedlings, 33,949 leaves, 468 leaf mines, 205 leaf galls, four leaf mine types (i.e., LM01, LM03, LM06 and LM09), and five leaf gall types (i.e., LG01, LG03, LG04, LG05 and LG06) were found in the investigation. The leaf miner species of LM01, LM03, LM06 were *Stigmella* sp. (Lepidoptera: Nepticulidae), *Tischeria* sp. (Tischeriidae), and *Acrocercops* sp. (Gracillariidae). LM09 and leaf gall species were not identified.

Numbers of *C. carlesii* seedlings in each 1-m² area were highest 0.5 m and 6.5 m from the road but lowest 3.5 m from the road; however, if a subset of squares with large seedling numbers only were considered, seedling numbers seemed to drop with the increasing distances (Figure 2a). Accumulated tree heights and numbers of leaves and of *C. carlesii* seedlings in each square decreased linearly against the distances from the road. Considering larger values of tree heights and leaf numbers only, the trends were also similar (Figure 2b,c).

The number of mines or galls in each seedling was linearly correlated with the number of leaves of the seedling ($r = 0.518$, $P < 0.001$; $r = 0.539$, $P < 0.001$ respectively). Therefore, the number of mines or galls per leaf should be used for the measurement of herbivory.

The number of mines per leaf in each square peaked at 0.5 m and dropped fluctuantly. When only large mine number values were considered, a decreasing trend could be noticed (Figure 2d). The number of galls per leaf in each square first peaked at 2 m and peaked again 5.5 m from road, a pattern that was con-

sistent with that of large gall number values (Figure 2e).

For different leaf miners, numbers of LM01, LM03, and LM09 per leaf peaked 0.5 m from the road and then dropped. The number of LM06 per leaf was very low at all distance classes and increased linearly but slowly against the distances from the road. Patterns of every leaf mine type were consistent to the variations of their large values (Figure 3).

For different leaf gallers, the number of LG01 per leaf peaked at about 2 m and 5.5 m from the road, which was similar to the pattern of total gall numbers. The number of LG03 per leaf peaked 5.5 m from the road. The number of LG04 and LG06 per leaf was very low at all distance classes, but LG04 numbers increased after 3.5 m from road, and LG06 numbers dropped from 0.5 m from road. The number of LG05 per leaf peaked about 1.5 m from the road and then dropped. Patterns of every leaf gall type were consistent to the variations of their large values (Figure 4).

Both Simpson's diversity index (D) and Shannon's diversity index (H) of leaf miners increased from 0.5 m to 4.5 m from road, and then H dropped (Figure 5), while D and H of leaf gallers decreased continuously with increasing distance from road (Figure 6).

Generally, the density of different leaf mines was ordered as follows: LM09 > LM01 > LM03 > LM06 (Figure 3); the density of different leaf galls was ordered as follows: LG05 > LG01 \approx LG03 > LG04 \approx LG06 (Figure 4).

Discussion

Generally, the number of individuals, tree heights, and the number of leaves of *C. carlesii* seedlings became lower with increased distance from road. These results

might indicate that old seedlings were fewer and the survival rate of seedlings was lower in forest interiors (Comita and Goldsmith 2008).

Leaf miners and leaf galls on *C. carlesii* seedlings showed different responses to the distance from the road. For example, leaf miners preferred the seedlings close to the forest road while leaf galls preferred the seedlings about 2 m away from the road. It is also interesting that species diversity of leaf miners was higher in the forest interior while species diversity of leaf galls was higher near the road. However, from the edge of the road to interior vegetation, both leaf miners and leaf galls generally decreased. Such patterns have been found for isopods, hemipterans, collembolans, sap-feeding herbivores, carabids, and carrion beetles (Muskett and Jones 1980; Lightfoot and Whitford 1991; Koivula 2005; Dunn and Danoff-Burg 2007).

For leaf miners, the number-distance patterns of LM01 (*Stigmella* sp.), LM03 (*Tischeria* sp.) and LM09 (an unknown leafminer) were similar to those of total leaf mines. However, the variations of LM03 at different distances were not so obvious. Contrarily, LM06 (another Gracillariid) only occurred in the forest interior (Figure 3). Seedlings suffered different damage levels from different leaf miners. There existed interspecific differences of the effects of the road among leaf miner species.

For leaf galls, seedlings endured different damage levels from different leaf galls. The influence of the road on LG01, LG03, LG04, LG05, and LG06 was different from species to species. Such interspecific differences between galling insects were also discovered in other studies (Martel 1995; Martinez and Wool 2006).

The ecological strategies of plants or animals are related to their distribution and behavior in accordance to the roads (Koivula 2005; Martinez and Wool 2006; Flory and Clay 2009; Avon et al. 2010). For example, insect species that prefer vigorous plants may choose roadside plants (Lightfoot and Whitford 1991; Price 1991) while insects using stressed plants may use plants under the vegetation cover (Martel 1995; Martinez and Wool 2006). Such theories might help to explain the varying effects of the road on different insect species in this study.

Since *C. carlesii* adults were dominant in the first tree layers, they must prefer the sun environment. Their seedlings might also grow better in more sunny conditions near the roads. According to another investigation on the distribution of leaf herbivory in a different canopy area of four Fagaceae tree species, including *C. carlesii*, in the same nature reserve, both leaf miners and leaf galls generally preferred sun leaves rather than shade leaves, while leaf galls had a lower degree of preference to sun than leafminers (Dai et al. 2013). These phenomena are consistent with this study: leaf mines were most abundant on leaves that were fully in the sun near roads, but leaf galls were not abundant on leaves that were fully in the sun, mostly occurring on leaves exposed to moderate sun a little farther away from the roads. For specific species, the mines of LM01 (*Stigmella* sp.) were mostly found in the leaves in the sun near the road, which is consistent with our previous observations (Dai et al. 2013) but contrary to other *Stigmella* (Nielsen and Ejlersen 1977; van Nieukerken et al. 2006). The mines of LM03 (*Tischeria* sp.) were more numerous in roadside sun leaves, but the trend was not distinct, which is similar to our other investigation (Dai et al. 2013). However, a *Tischeria* sp. has no preferences between sun leaves or shade

leaves of *Quercus emoryi* (Bultman and Faeth 1988; Connor 2006). The mines of LM09 (an unknown leafminer) occurred only in young leaves, which were more in roadside plant individuals. Some gracillariid moths also preferred shade leaves (Nielsen and Ejlersen 1977; Faeth 1991a; Faeth 1991b; Angulo-Sandoval and Aide 2000), just like LM06 (another Gracillariid miner).

Acknowledgements

We thank Ren-Lin Liu for plant identification, Jin-Shui Liang, Si-Min Li, Xing-Lu Ding, and Si-Si Wei for field investigations, Pei-Rong Tang, Hua Zhou, and Jiulianshan National Nature Reserve for field assistance, Robert Anderson, E. J. van Nieukerken, Hou-Hun Li, Hai-Yan Bai, Charles L Bellamy, and Jonas Rimantas Stonis for mine identification. We also thank Nadir Erbilgin for the improvement of our manuscript. This study was financially supported by The National Natural Science Foundation of China (31070341, 31260116), The Science and Technology Foundation for Selected Overseas Chinese Scholars, Ministry of Human Resources and Social Security, The Science and Technology Foundation of Jiangxi Educational Department (GJJ13663), The Jiangxi Province Natural Science Foundation of China (20132BAB204008), and The Young Scientists Training Plan of Jiangxi Province, China (20133BCB23026) to X. Dai.

References

- Angold P. 1997. The impact of a road upon adjacent heathland vegetation: effects on plant species composition. *Journal of Applied Ecology* 34: 409-417.
- Angulo-Sandoval P, Aide TM. 2000. Effect of plant density and light availability on leaf damage in *Manilkara bidentata* (Sapotaceae). *Journal of Tropical Ecology* 16: 447-464.
- Avon C, Bergès L, Dumas Y, Dupouey JL. 2010. Does the effect of forest roads extend a few meters or more into the adjacent forest? A study on understory plant diversity in managed oak stands. *Forest Ecology and Management* 259(8): 1546-1555.
- Bultman TL, Faeth SH. 1988. Abundance and mortality of leaf miners on artificially shaded Emory oak. *Ecological Entomology* 13(2): 131-142.
- Comita LS, Goldsmith GR. 2008. Impact of research trails on seedling dynamics in a tropical forest. *Biotropica* 40(2): 251-254.
- Connor EF. 2006. Effects of the light environment on oviposition preference and survival of a leaf-mining moth, *Cameraria hamadryadella* (Lepidoptera : Gracillariidae), on *Quercus alba* L. *Ecological Entomology* 31(2): 179-184.
- Dai X, Xu J, Ding X. 2013. Circular distribution pattern of plant modulars and endophagous herbivory within tree crowns: The impact of roadside light conditions *Journal of Insect Science* 13:141. Available online: www.insectscience.org/13.141
- Dai X, Zhu C, Xu J, Liu R, Wang X. 2011. Effects of physical leaf features of host plants on leaf-mining insects. *Acta Ecologica Sinica* 31(5): 1440-1449.
- De Boor C. 2001. *A practical guide to splines*. Springer Verlag.
- Dunn RR, Danoff-Burg JA. 2007. Road size and carrion beetle assemblages in a New York

forest. *Journal of Insect Conservation* 11(4): 325-332.

Faeth SH. 1991a. Effect of oak leaf size on abundance, dispersion and survival of the leafminer *Cameraria* sp. (Lepidoptera: Gracillariidae). *Environmental Entomology* 20(1): 196-204.

Faeth SH. 1991b. Novel aspects of host tree resistance to leafminers. In: Baranchikov YN, Mattson WJ, Hain FP, Payne TL, Editors. *Forest Insects Guilds: Patterns of Interaction with Host Trees*. USDA Forest Service, Gen. Tech. Rep. NE-153: 219-239.

Flory SL, Clay K. 2006. Invasive shrub distribution varies with distance to roads and stand age in eastern deciduous forests in Indiana, USA. *Plant Ecology* 184(1): 131-141.

Flory SL, Clay K. 2009. Effects of roads and forest successional age on experimental plant invasions. *Biological Conservation* 142(11): 2531-2537.

Forman RTT, Alexander LE. 1998. Roads and their major ecological effects. *Annual Review of Ecology and Systematics* 29: 207-231+C202.

Godefroid S, Koedam N. 2004. The impact of forest paths upon adjacent vegetation: effects of the path surfacing material on the species composition and soil compaction. *Biological Conservation* 119(3): 405-419.

Hammer Ø, Harper D, Ryan P. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica* 4(1): 9.

Hastie TJ, Tibshirani RJ. 1990. *Generalized additive models*. Chapman & Hall/CRC.

Koivula MJ. 2005. Effects of forest roads on spatial distribution of boreal carabid beetles (Coleoptera: Carabidae). *The Coleopterists Bulletin* 59(4): 465-487.

Krassilov V. 2008. Mine and gall predation as top down regulation in the plant-insect systems from the Cretaceous of Negev, Israel. *Palaeogeography Palaeoclimatology Palaeoecology* 261(3-4): 261-269.

Lightfoot DC, Whitford WG. 1991. Productivity of creosotebush foliage and associated canopy arthropods along a desert roadside. *American Midland Naturalist* 125(2): 310-322.

Liu XZ, Xiao ZY, Ma JH. 2002. *Scientific Survey and Study on the Forest Ecosystem in Jiulianshan Nature Reserve*. China Forestry Press.

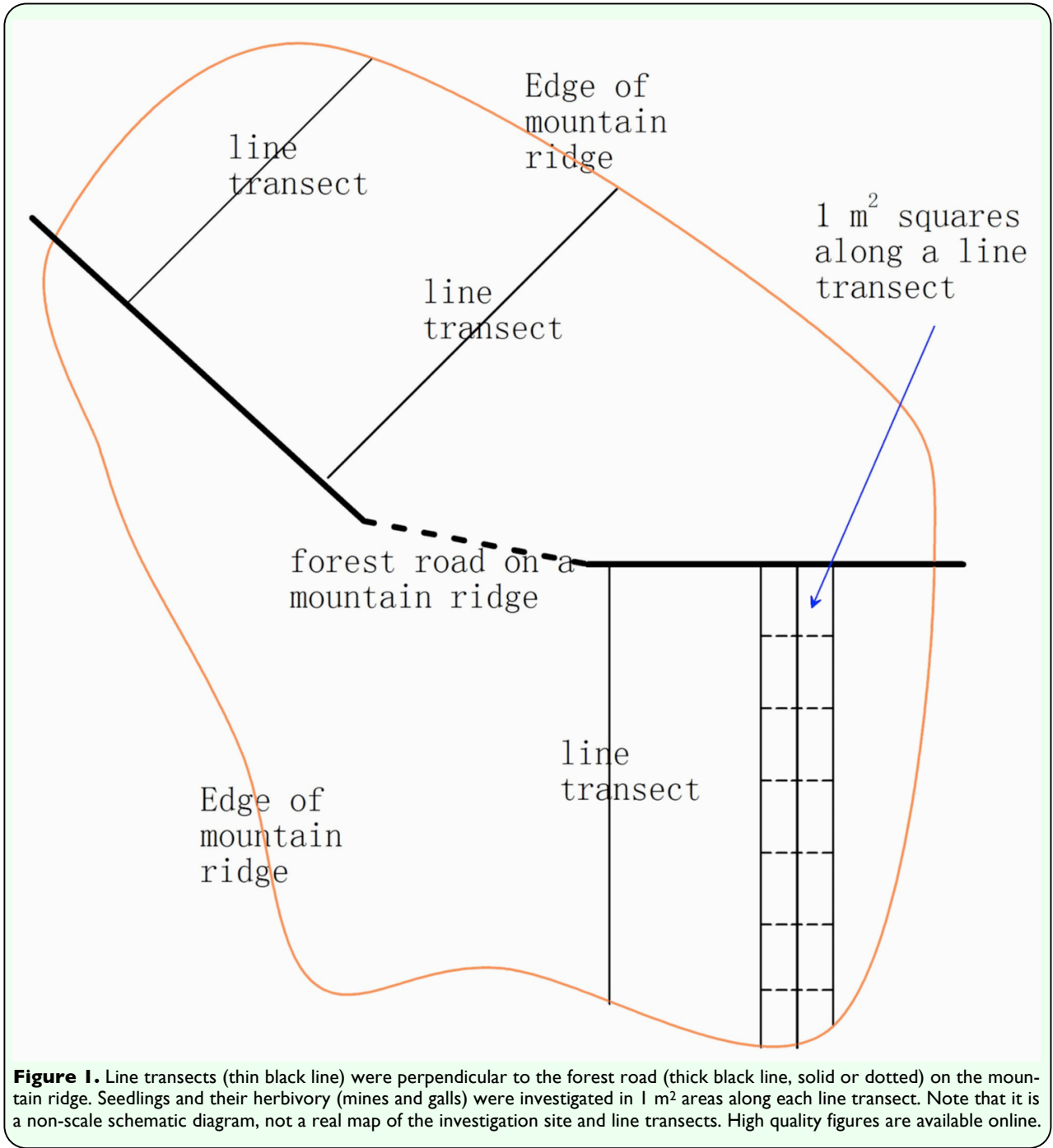
Marsh DM, Beckman NG. 2004. Effects of forest roads on the abundance and activity of terrestrial salamanders. *Ecological Applications* 14(6): 1882-1891.

Martel J. 1995. Performance of *Eurosta solidaginis* (Diptera: Tephritidae) and *Epiblema scudderiana* (Lepidoptera: Tortricidae), two gall-formers of goldenrod, in roadside environments. *Environmental Entomology* 24(3): 697-706.

Martinez JJI, Wool D. 2006. Sampling bias in roadsides: the case of galling aphids on Pistacia trees. *Biodiversity and Conservation* 15(7): 2109-2121.

Muskett C, Jones M. 1980. The dispersal of lead, cadmium and nickel from motor vehicles and effects on roadside invertebrate macrofauna. *Environmental Pollution Series A, Ecological and Biological* 23(3): 231-242.

- Nielsen BO, Ejlersen A. 1977. The distribution pattern of herbivory in a beech canopy. *Ecological Entomology* 2(4): 293-300.
- Ortega YK, Capen DE. 2002. Roads as edges: effects on birds in forested landscapes. *Forest Science* 48(2): 381-390.
- Price PW. 1991. The plant vigor hypothesis and herbivore attack. *Oikos* 62: 244-251.
- Reijnen R, Foppen R, Braak CT, Thissen J. 1995. The effects of car traffic on breeding bird populations in woodland. III. Reduction of density in relation to the proximity of main roads. *Journal of Applied Ecology* 32(1): 187-202.
- Rokaya MB, Münzbergová Z, Shrestha MR, Timsina B. 2012. Distribution patterns of medicinal plants along an elevational gradient in central Himalaya, Nepal. *Journal of Mountain Science* 9(2): 201-213.
- Spencer H, Port G. 1988. Effects of roadside conditions on plants and insects. II. Soil conditions. *Journal of Applied Ecology* 25(2): 709-714.
- Spencer H, Scott N, Port G, Davison A. 1988. Effects of roadside conditions on plants and insects. I. Atmospheric conditions. *Journal of Applied Ecology* 25(2): 699-707.
- van Nieukerken EJ, Schreurs AEP, van Stiphout ML, Ellis WN. 2006. *Stigmella aceris* (Lepidoptera : Nepticulidae), a new leafminer of maples in The Netherlands and Belgium. *Entomologische Berichten (Amsterdam)* 66(6): 174-180.
- Wang H, Cai Y, Li K, Jiang H, Tian Y. 2006. Insect herbivory patterns on leaves of 11 plant species in the evergreen broad-leaved forests of Tiantong National Forest Park, Zhejiang. *Biodiversity Science* 14(2): 145-151.
- Watkins RZ, Chen J, Pickens J, Brosnokske KD. 2003. Effects of forest roads on understory plants in a managed hardwood landscape. *Conservation Biology* 17(2): 411-419.



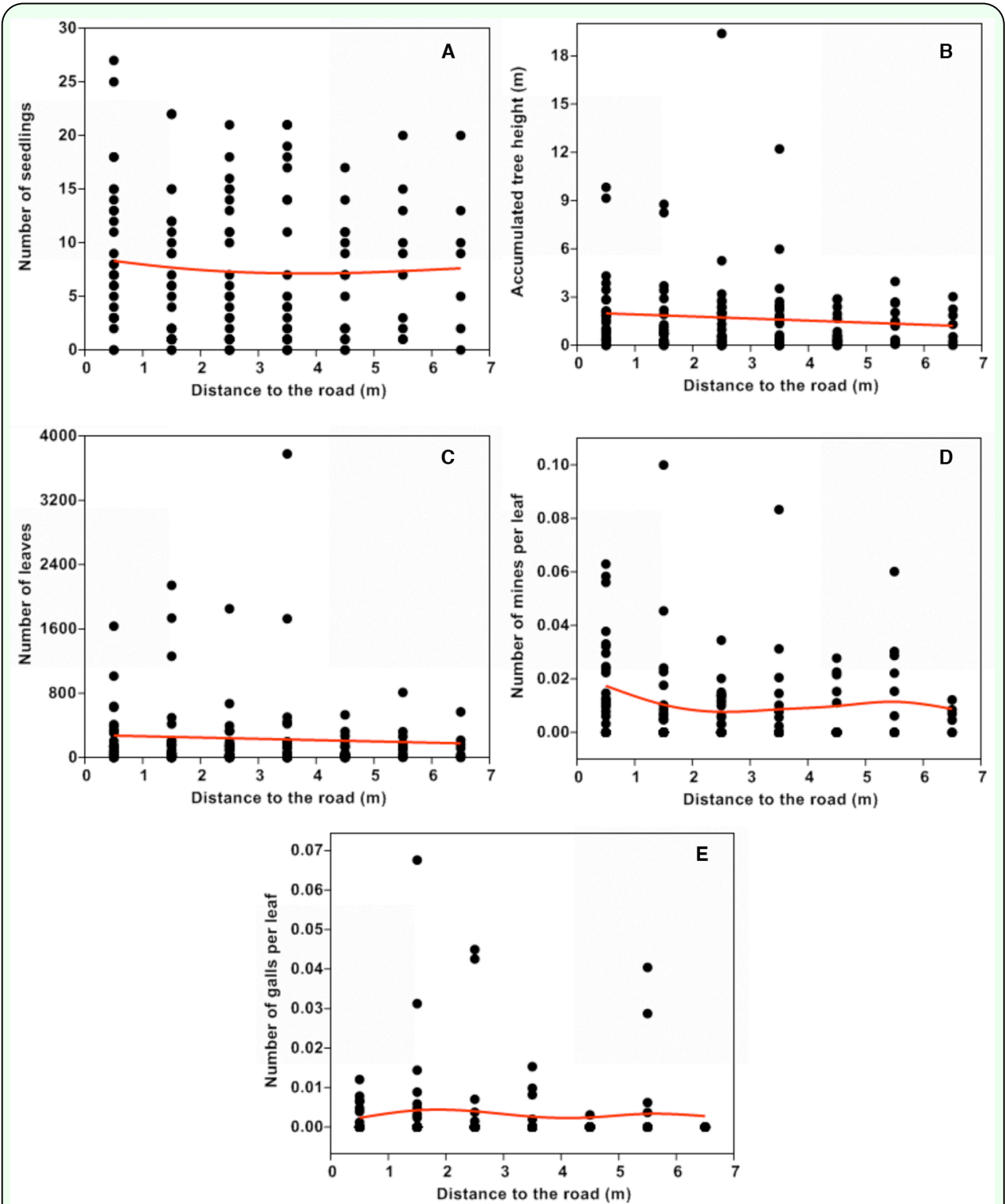


Figure 2. Scatterplots of number of seedlings (a), accumulated tree height (b), number of leaves (c), number of mines per leaf (d), and number of galls per leaf (e) against distance from road. The line was fitted by generalized additive models with a third-order polynomial smoothing spline. High quality figures are available online.

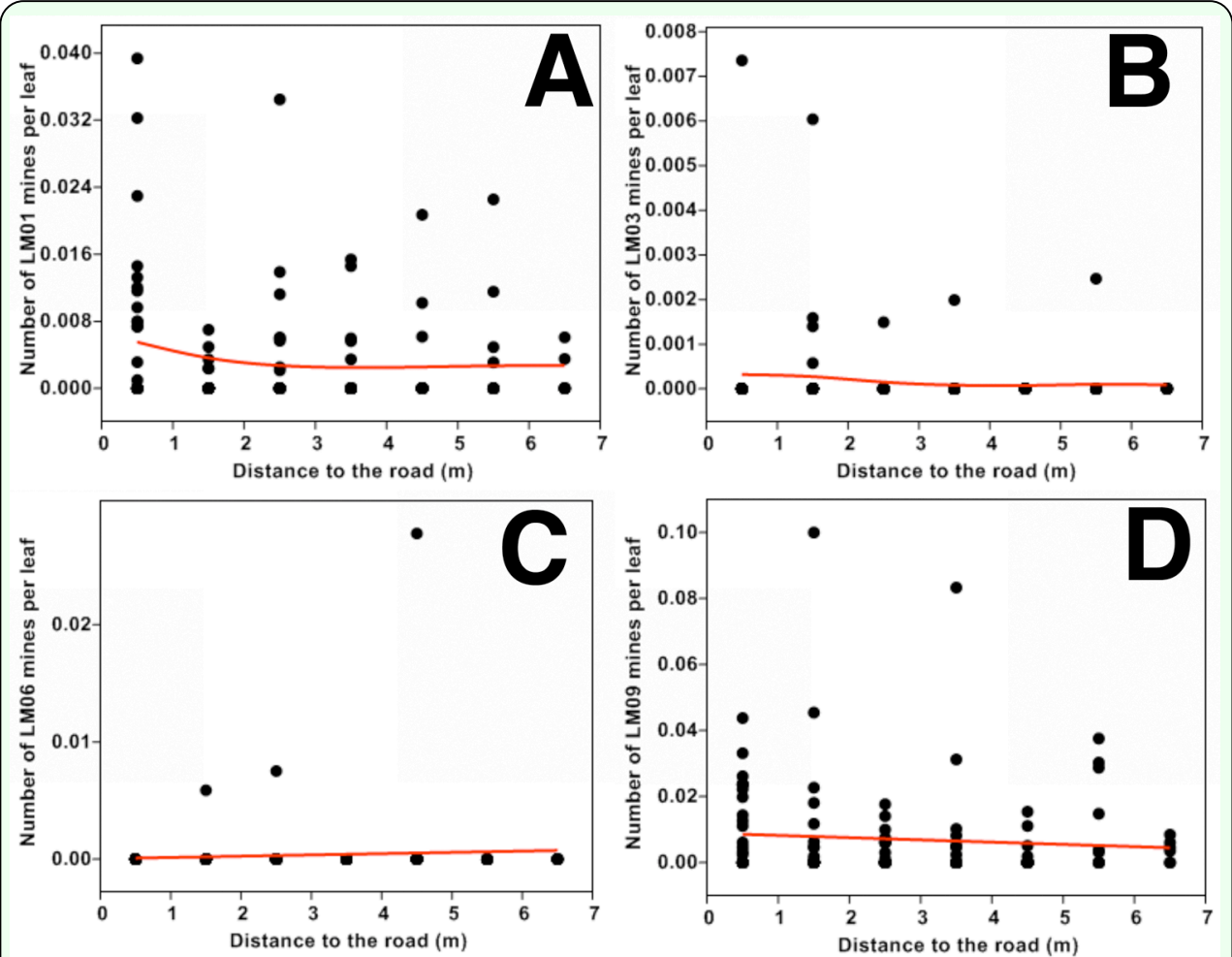


Figure 3. Scatterplots of number of different leaf mine types per leaf against distance from road: LM01 (a), LM03 (b), LM06 (c), and LM09 (d). The line was fitted by generalized additive models with a third-order polynomial smoothing spline. High quality figures are available online.

