Effects of host plant growth form on dropping behaviour in leaf beetles

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Many leaf-eating insects drop from their host plants to escape predators. However, they must return to the leaves of the host plant after dropping, which represents a cost associated with this behaviour. In woody plants, the positioning of leaves is generally higher than that of herbaceous plants, which suggests that dropping from woody plants might be costlier for leaf-eating insects than dropping from herbaceous plants. Therefore, we predicted that dropping behaviour would be observed less frequently in insects that feed on woody plant leaves than in those that feed on herbaceous plant leaves. To test this prediction, we investigated dropping behaviour experimentally in larvae (23 species) and adults (112 species) of leaf beetles (Coleoptera: Chrysomelidae) on their host plants (86 species of 44 families) in field conditions. Larvae on woody plants exhibited dropping behaviour less frequently than those on herbaceous plants. However, this pattern was not detected in adults. Thus, host plant growth form might affect the evolution of dropping behaviour in leaf beetle larvae, but not in winged adults, perhaps owing to their higher mobility.

ADDITIONAL KEYWORDS:   adults – anti-predator defences – behavioural costs – chemical defences – Chrysomelidae – Coleoptera – herbaceous plants – larvae – woody plants.

INTRODUCTION

Animals defend themselves against predators in various ways (Edmunds, 1974; Sugiura, 2020). The evolution of anti-predator defences can be driven by benefits gained from protection against predators and by the associated costs (Bowers, 1992; Camara, 1997; Zevereva et al., 2017). The benefits and costs of defences are closely associated with habitat. For example, phytophagous insects sequester host plant chemicals in their bodies to defend against predators (Nishida, 2002); the effectiveness of chemical defences can vary among host plant species because they have different enemies and access to different chemical components (Denno et al., 1990; Singer & Stireman, 2003). Although studies have investigated the effects of host plants on the effectiveness of anti-predator defences (Denno et al., 1990; Singer & Stireman, 2003), few studies have clarified the effects of host plants on the costs associated with anti-predator defences in phytophagous insects (Matsubara & Sugiura, 2018).

Dropping behaviour for rapid escape from predators is among the simplest of anti-predator defences (Humphreys & Ruxton, 2019). This behaviour has been reported in various animal groups, such as mammals, lizards and insects (Edmunds, 1974; Lima, 1993; Losey & Denno, 1998a, b; Vitt et al., 2002; Martins et al., 2005; Sato et al., 2005; Castellanos et al., 2011; Greeney et al., 2012; Barnett et al., 2015, 2017; Humphreys & Ruxton, 2019; Sugiura, 2020). Therefore, dropping behaviour has evolved convergently in diverse animal groups (Humphreys & Ruxton, 2019). Dropping is frequently associated with other defensive behaviours; for example, some phytophagous insects feign death (i.e. exhibit thanatosis) as they drop from host plants (Ohno & Miyatake, 2007; Matsubara & Sugiura, 2018). Although dropping allows rapid escape from predators (Day et al., 2006; Francke et al., 2008), sudden departure from the food resources and microhabitat can reduce feeding efficiency and reproduction and increase development time (Roitberg et al., 1979; Loughridge & Luff, 1983; Dill et al., 1990; Losey & Denno, 1998c; Nelson, 2007; Perović et al., 2008; Agabiti et al., 2016). These are considered to be costs associated with dropping behaviour.
Host plants can affect the cost of dropping behaviour in phytophagous insects (Matsubara & Sugiura, 2018). When phytophagous insects drop to the ground, they must then return to an appropriate feeding position on the host plant. Underlying leaves can prevent phytophagous insects from dropping to the ground, thereby reducing the costs of dropping behaviour. For example, leaf beetles are much less likely to drop to the ground from large, oval leaves than from cleft leaves because the former can act as safety nets for the falling beetles (Matsubara & Sugiura, 2018). Given that the leaf positions on woody plants are generally higher than those on herbaceous plants, insects require more time and energy to move from the ground to their feeding position on woody plants. Consequently, the costs associated with dropping from woody plants are expected to be higher than those associated with dropping from herbaceous plants. Some arthropods that inhabit woody plants reportedly avoid dropping to the ground by gliding (Yanoviak et al., 2005, 2009, 2015; Meresman et al., 2017) or using silk lifelines (Brackenbury, 1996; Sugiura & Yamazaki, 2005). Therefore, dropping behaviour is expected to occur less frequently in insects that feed on woody plant leaves than in those that feed on herbaceous plant leaves. However, this prediction has never been tested. Clarification of the relationships between host plants and dropping behaviour would contribute to our understanding of the evolution of defensive behaviours and host plant selection in phytophagous insects.

To elucidate the effects of host plant growth form (i.e. herbaceous vs. woody plants) on dropping behaviour in phytophagous insects, we investigated the anti-predator defences of leaf beetles (Coleoptera: Chrysomelidae) on their host plants in field conditions in Japan. The species diversity of leaf beetles is high; the family Chrysomelidae includes ~41 000 extant species (Jolivet et al., 2008), ~700 of which are recorded in Japan (Kimoto & Takizawa, 1994). Most leaf beetle species are dietary specialists that feed on particular families and genera of plants (Kimoto & Takizawa, 1994; Jolivet & Verma, 2002). Although closely related beetle species frequently use the same plant families, some congenic species feed on different plant families (Kimoto & Takizawa, 1994). Leaf beetles defend themselves against predators in various ways, including dropping, flying, jumping, clinging, having spines and self-mimicking of feeding damage (Kimoto & Takizawa, 1994; Jolivet & Verma, 2002; Konstantinov et al., 2018; Shinohara & Takami, 2020; Sugiura, 2020). The secretion of chemical liquids from the body is well documented in some leaf beetle larvae as a chemical defence against predators such as ants (Sugawara et al., 1979; Pasteels et al., 1982; Pasteels et al., 1984; Kimoto & Takizawa, 1994). Thus, leaf beetles are an appropriate insect group for investigating the effects of host plant growth form on dropping behaviour and other anti-predator defences in phytophagous insects. Larvae and adults of many leaf beetle species are found on host plant leaves, making it possible to compare the effects of host plant growth form on dropping behaviour in larvae and adults.

In this study, we investigated experimentally the defensive behaviour of larvae (23 species) and adults (112 species) of leaf beetles on their host plants in field conditions in Japan. We examined whether larvae and adults showed dropping or other defensive behaviours in response to artificial stimuli on their host plants and explored the effects of host plant growth form (herbaceous or woody plants) on the evolution of dropping behaviour in leaf beetles.

MATERIAL AND METHODS

STUDY SITE AND SPECIES

Field experiments were conducted at 102 sites in Japan (Matsubara & Sugiura, 2021; 25°51′–41°22′N, 127°42′–141°22′E, 1–1090 m a.s.l.). The study sites included various environments, such as forest, grassland and farm. Leaf beetle larvae are external leaf feeders, leaf/stem miners, seed borers, detritus feeders or root-feeders (Jolivet, 1988). In this study, we focused on external leaf feeders that potentially drop from host plant leaves. Early instar larvae were not included in this study because they were too small to be investigated in field conditions. Therefore, larvae with body length ≥ 5 mm were used for subsequent experiments.

Leaf beetles and their host plants were identified based on their morphological characteristics (Kimoto & Takizawa, 1994; Azegami et al., 2013a, b; Hayashi, 2014). The growth form (woody or herbaceous) of each plant species was determined based on the presence or absence of woody tissues in the stems (Clapham et al., 1987; Azegami et al., 2013a, b; Hayashi, 2014).

FIELD EXPERIMENTS

To explore the effects of host plant growth form (woody or herbaceous) on the defensive behaviours of leaf beetles, we investigated the responses of beetles to simulated attacks in field conditions, following the procedure of Matsubara & Sugiura (2018). When larval or adult leaf beetles fed externally on plant leaves, we measured the feeding elevation (i.e. vertical distance from the ground to the feeding position). Individuals on leaves higher than 3.0 m were not included in our experiments because they could not be reached. We randomly selected beetles on leaves (height, ≤ 3.0 m) and poked the larval dorsal abdomen or adult elytra with forceps to simulate attacks by predacious insects.
DROPPING BEHAVIOUR IN LEAF BEETLES

(e.g. ants). Forceps have been used frequently to simulate predator attacks (e.g. Miyatake et al., 2008; Müller et al., 2016; Matsubara & Sugiura, 2018). To standardize the stimuli, the same researcher used the same forceps in all the field experiments. The same degree of stimulus was provided to all leaf beetles in this study. When beetles did not respond to a stimulus, they were poked repeatedly at 2 s intervals, up to five times. Beetles on the lowest leaves are more likely to drop to the ground than those on the overlying leaves, because underlying leaves can prevent beetles from reaching the ground (Matsubara & Sugiura, 2018). Therefore, to evaluate the effects of underlying leaves on dropping behaviour, we did not use beetles found on the lowest leaves in our experiments.

Initially, we recorded whether beetles exhibited dropping behaviour in response to artificial stimuli. Dropping behaviour was defined as departure from the feeding position attributable to gravity. When insects dropped from the host plant leaves, we also recorded the place to which they dropped (i.e. leaves or ground/water) or whether they flew before landing (Fig. 1). Dropping behaviour is frequently accompanied by thanatosis (Humphreys & Ruxton, 2018); for example, some adults of a leaf beetle species fold their legs and antennae to feign death as they drop from host plants (Matsubara & Sugiura, 2018). We included such death feigning as dropping behaviour in this study, because it was difficult to observe whether each leaf beetle feigned death during dropping. We also recorded other defensive behaviours, such as emission of visible liquids, flying, jumping and running.

Field experiments were conducted on sunny or cloudy days (08:30–18:00 h) from April 2016 to May 2020, at temperatures of 16.2–35.4 °C.

DATA ANALYSIS

The defensive behaviours of some beetle species were observed at several sites. However, site effects were not included in following models because our preliminary analysis detected no site effects on dropping behaviour.

Generalized linear mixed models (GLMMs) with binomial error distribution and logit link were used to determine the effects of host plant growth form on dropping and other behaviours of leaf beetles. As response variables, we used whether each individual exhibited dropping behaviour or not (one or zero), whether each individual dropped to the ground or not (one or zero), whether each larva exhibited chemical defence or not (one or zero) and whether each adult flew during dropping or not (one or zero). In all analyses, host plant growth form (woody or herbaceous) was used as a fixed factor. Leaf beetle tribe and species were fitted as nested random effects to account for phylogenetic constraints (Hiraiwa & Ushimaru, 2017).

Figure 1. Four patterns of dropping behaviour in leaf beetles: A, dropping to the same leaf; B, dropping to an underlying leaf; C, dropping to the ground or water; and D, flying during during dropping.
All analyses were performed using the software R v.3.5.2 \citep{RCoreTeam2018}. The GLMMs were run using the \texttt{lme4} package 1.1.13 \citep{Bates2017}.

**RESULTS**

A total of 286 leaf beetle larvae (23 species from six tribes) on host plants (23 species from 16 families) were investigated in field conditions; 15 and eight beetle species fed on the leaves of woody and herbaceous plants, respectively \citep{Appendix1}. The larval feeding elevations were 1501.2 ± 36.9 mm (mean ± SEM, \(N = 186\)) on woody plants and 315.6 ± 14.1 mm (\(N = 100\)) on herbaceous plants. When poked with forceps, 2.2% of 186 larvae (13.3% of 15 species) on woody plants and 36.0% of 100 larvae (87.5% of eight species) on herbaceous plants exhibited dropping behaviour (Fig. 2A; Table 1). Larvae on herbaceous plants dropped to the ground more frequently compared with those on woody plants (Fig. 2B; Table 1). Other defensive behaviours were also observed. For example, 52.2% of 186 larvae (60.0% of 15 species) on woody plants and 56.0% of 100 larvae (87.5% of eight species) on herbaceous plants secreted visible chemicals, with no significant difference between woody and herbaceous plants (Fig. 2C; Table 1). Other larvae (1.4%) raised their faecal shields in response to artificial stimuli.

A total of 809 leaf beetle adults (112 species from 22 tribes) on host plants (86 species from 44 families) were investigated; 57 and 55 beetle species fed on the leaves of woody and herbaceous plants, respectively \citep{Appendix1}. The feeding elevations of adult beetles were 1207.7 ± 26.9 mm (\(N = 393\)) on woody plants and 524.6 ± 18.5 mm (\(N = 416\)) on herbaceous plants. When poked with forceps, 37.7% of 393 leaf beetle adults (64.9% of 57 species) on woody plants and 39.2% of 416 adults (58.2% of 55 species) on herbaceous plants exhibited dropping behaviour (Fig. 3A; Table 2). Host plant growth form did not significantly affect dropping behaviour in leaf beetle adults (Fig. 3A, B; Table 2). In addition, significantly more adults flew after dropping from woody (9.4%) than from herbaceous plants (2.9%; Fig. 3C; Table 2). Beetles that did not drop ran (22.1%), jumped (14.2%), flew (7.3%) or secreted visible chemical liquids from their bodies (0.6%) in response to stimuli. Other beetles (1.9%) used their legs to hold tenaciously to leaf surfaces.

**DISCUSSION**

Previous studies have investigated the effects of host plants on anti-predator defences in phytophagous insects \citep{Denno1990}. However, few studies have focused on host plant architecture as a factor that affects dropping behaviour in leaf-feeding insects \citep{Matsubara2018}. In the present study, we conducted field experiments to test the prediction that dropping behaviour would be observed less frequently among
Leaf beetle larvae exhibited dropping behaviour less frequently than those on herbaceous plants (Fig. 2A; Table 1), supporting our prediction. However, this pattern was not detected in adults (Fig. 3A; Table 2).

**Defensive Strategies in Leaf Beetles**

Leaf beetle larvae exhibited chemical defence more frequently than dropping behaviour in our experiments (Fig. 2C; Table 1). Thus, larvae frequently avoided leaving their feeding sites. Most larvae that dropped from host plants ultimately landed on the ground (Fig. 2B), necessitating a return to the host plant leaves by walking. Given that leaf positions on woody plants are generally higher than those on herbaceous plants, dropping from woody plants might be costlier compared with dropping from herbaceous plants. Therefore, larvae that feed on woody plants are generally less frequent than those on herbaceous plants (Fig. 2A). However, larvae that feed on woody plants were more likely to avoid leaving their feeding sites than those that feed on herbaceous plants (Fig. 2A). However, larvae that feed on woody plants did not use chemical defences more frequently than those that feed on herbaceous plants (Fig. 2C). Given that chemicals emitted by leaf beetle larvae can effectively repel predators such as ants and spiders (Sugawara et al., 1979; Pasteels et al., 1982, 1988; Kimoto & Takizawa, 1994), the larvae of many leaf beetle species prefer to adopt chemical defences. However, we might have overestimated the importance of chemical defences in larval leaf beetles because our study was limited to the larvae of 23 species (six tribes of Chrysomelidae).

Leaf beetle adults exhibited dropping behaviour more frequently than larvae (Figs 2A, 3A). However, the drop rates of adult beetles did not differ significantly between woody and herbaceous plants (Fig. 3A; Table 2). Dropped adults frequently returned to host plant leaves by flying before landing on the ground, suggesting that the costs associated with dropping behaviour are lower for adults that can fly. The results of our previous study showed that larvae of the leaf beetle *Phaedon brassicae* Baly required more time than did adults to return to feeding sites (i.e. leaves) on host plants (Matsubara & Sugiura, 2018). Given that the costs associated with dropping to the ground are lower for adults than for larvae, adults might not avoid leaving their host plants in response to predator attacks. Alternative defensive behaviours of adults included running, jumping and flying. Although very few adults emitted visible chemical liquids from their bodies, adults of some leaf beetle species reportedly secrete small amounts of defensive chemicals (Pasteels et al., 1988). Thus, our study might have underestimated the importance of chemical defences in adult leaf beetles.

**Effects of Plant Growth Form on Dropping Behaviour**

We observed effects of host plant growth form (i.e. woody or herbaceous plants) on dropping behaviour.
Dropping behaviour in leaf beetle larvae might have evolved via at least one of four potential processes: (1) species that feed on herbaceous plants acquiring dropping behaviour; (2) species that feed on woody plants losing dropping behaviour; (3) species that exhibit dropping behaviour shifting from woody to herbaceous host plants; or (4) species that do not exhibit dropping behaviour shifting from herbaceous to woody host plants. The drop rate can vary among larvae of the same leaf beetle species (Appendix 1; Matsubara & Sugiura, 2018), which suggests that this behaviour is frequently acquired or lost among species (i.e. hypothetical process 1 or 2). We did not observe whether oviposition site preferences (e.g. woody or herbaceous plants) varied among adults of the same leaf beetle species in the present study. However, oviposition preferences for host plant species reportedly vary among adult females of the same leaf beetle species (Vencl & Srygley, 2013; Vencl et al., 2011, 2013), suggesting that shifts in host plant occur frequently among species (i.e. hypothetical process 3 or 4). Molecular phylogenetic analyses (e.g. ancestral reconstruction) of defensive behaviours and host plants would help to elucidate the selective processes promoting the evolution of dropping behaviour in leaf beetles.

**CONCLUSION**

The results of this study indicate that host plant growth form affected the evolution of dropping behaviour in leaf beetle larvae but not in winged adults. However, the evolution of dropping behaviour in phytophagous insects can be influenced by other factors, such as host plant range. When generalist species drop from the host plant to the ground, they can find other plants to eat more easily than can specialist species (Bernays & Graham, 1988). Therefore, the costs associated with dropping from host plants might be higher for specialists than for generalists. In addition, primary defences, such as body colour, might affect the evolution of dropping behaviour in phytophagous insects. Insect species with cryptic body colour are less easily detected by predators compared with those having aposematic body colour when they drop to the ground. Consequently, dropping from host plants might have evolved more frequently in cryptic species than in aposematic species. Further studies are needed to test these effects on the evolution of dropping behaviour in phytophagous insects.

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Figure 3. Dropping and other defensive behaviours of leaf beetle adults. Proportions of leaf beetle adults: A, exhibiting dropping behaviour; B, dropping to the ground; C, flying during dropping. Sample size: 393 individuals (57 species) on woody plants and 416 individuals (55 species) on herbaceous plants.

in leaf beetle larvae (Fig. 2A) but not in the adults (Fig. 3A). Given that few leaf beetle larvae can move among host plants, host plants can strongly influence their survival. The costs associated with dropping from woody plants might be higher than those associated with dropping from herbaceous plants, and differential costs between woody and herbaceous plants should be higher in leaf beetle larvae than in flying adults.

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**SHARED DATA**

The data from the study are available from the Figshare Digital Repository (Matsubara & Sugiura, 2021).
### Appendix 1. Host plants and dropping behaviour of leaf beetles used in this study

| Subfamily | Code | Tribe | Species          | Dropping* | Type† | Host plants          | Growth form |
|-----------|------|-------|------------------|-----------|-------|----------------------|-------------|
| Alticinae |      |       |                  |           |       |                      |             |
|           | 1    | Alticini | Altica caerulescens | 7 (7)     | ELF   | Acalypha australis   | Herbaceous  |
|           | 2    | Alticini | Altica cyanea     | 0 (10)    | ELF   | Ludwigia epilobioides | Herbaceous  |
|           | 3    | Alticini | Altica oleracea   | 3 (4)     | ELF   | Trapa japonica       | Herbaceous  |
|           | 4    | Alticini | Altica sp.        | 0 (1)     | RF    | Isodon sp.           | Herbaceous  |
|           | 5    | Alticini | Aphthona formosana | 1 (8)     | RF    | Mallotus japonicus   | Woody       |
|           | 6    | Alticini | Aphthona perminuta | 0 (4)     | RF    | Castanea crenata     | Woody       |
|           | 7    | Alticini | Aphthonalitica angustata | 4 (10) | UK    | Akebia trifoliata    | Woody       |
|           | 8    | Alticini | Argopus balyi     | 0 (2)     | LM    | Clematis apiifolia   | Herbaceous  |
|           | 9    | Alticini | Chaetocnema sp.   | 2 (3)     | RF    | Digitaria ciliaris   | Herbaceous  |
|           | 10   | Alticini | Clitea metallica  | 0 (5)     | ELF   | Citrus unshiu        | Woody       |
|           | 11   | Alticini | Epitrix hirtipennis | 0 (10)  | RF    | Solanum melongena    | Herbaceous  |
|           | 12   | Alticini | Hemipyxis cinctipes | 0 (11)  | ELF   | Clerodendrum trichotomum | Woody |
|           | 13   | Alticini | Hemipyxis flavipennis | 2 (3)  | ELF   | Clematis apiifolia   | Herbaceous  |
|           | 14   | Alticini | Langa fulva       | 0 (1)     | UK    | Piper kadsura        | Woody       |
|           | 15   | Alticini | Longitarus scutellaris | 0 (5) | LF    | Plantago asiatica    | Herbaceous  |
|           | 16   | Alticini | Longitarus sp.    | 0 (10)    | ELF   | Heliotropium arborescens | Woody |
|           | 17   | Alticini | Mantura clavareau | 0 (5)     | LM    | Rumex acetosa        | Herbaceous  |
|           | 18   | Alticini | Nonarthra cyanea  | 0 (2)     | UK    | Cerasus x yedoensis  | Woody       |
|           | 19   | Alticini | Philopona vibex   | 0 (2)     | ELF   | Plantago asiatica    | Herbaceous  |
|           | 20   | Alticini | Phyllotreta striolata | 0 (15) | RF    | Brassica rapa        | Herbaceous  |
|           | 21   | Alticini | Psylliodes punctifrons | 0 (10) | RF    | Brassica rapa        | Herbaceous  |
|           | 22   | Alticini | Sphaerodermia nigricolle | 2 (8) | LM    | Smilax china         | Woody       |
|           | 23   | Alticini | Sphaerodermia quadrimaculatum | 0 (8) | LM    | Clematis apiifolia   | Herbaceous  |
|           | 24   | Alticini | Sphaerodermia tarsatum | 0 (10) | LM    | Sasa kurilensis      | Herbaceous  |
|           | 25   | Alticini | Sphaerodermia unicolor | 0 (8)  | LM    | Clematis terniflora  | Herbaceous  |
| Bruchinae |      |       |                  |           |       |                      |             |
|           | 26   | Amblycerini | Spermaphagopus rufiventris | 0 (2) | SEB   | Ipomoea indica      | Herbaceous  |
| Cassidinae | 27  | Aspidimorphini | Laccoptera quadrimaculata | 0 (8) | LF    | Ipomoea cairica      | Herbaceous  |
|           | 28   | Cassidini | Cassida circumdata | 2 (6)     | LM    | Ipomoea indica       | Herbaceous  |
|           | 29   | Cassidini | Cassida nebulosa   | 0 (1)     | ELF   | Cheno podium album    | Herbaceous  |
|           | 30   | Cassidini | Cassida piperata   | 0 (3)     | ELF   | Amaranthus blitum    | Herbaceous  |
|           | 31   | Cassidini | Cassida versicolor | 0 (6)     | ELF   | Cerasus x yedoensis  | Woody       |

* Dropping refers to the frequency of dropping behaviour during the study.
† Type: ELF = Early Larval Feeding, RF = Larval Feeding, LM = Late Larval Feeding, SEB = Seed Feeding, UK = Unknown.
### Appendix 1. Continued

| Code | Subfamily | Tribe | Species | Dropping* | Type† | Host plants |
|------|-----------|-------|---------|-----------|-------|-------------|
|      |           |       |         | Adult | Larva | Species            | Growth form |
| 32   | Cassidini | Cassida vespertina | 0 (1) | –   | ELF | Clematis apiifolia | Herbaceous    |
| 33   | Cassidini | Thlaspida cribrosa | 0 (9) | 0 (8) | ELF | Callicarpa japonica | Woody         |
| 34   | Cassidini | Thlaspida levisi | 0 (1) | –   | ELF | Praxinus lanuginosa | Woody         |
| 35   | Hispini   | Daelysia subquadra | 2 (3) | –   | LM  | Castanea crenata | Woody         |
| 36   | Hispini   | Rhadina nigrocyanea | 4 (11) | –   | LM  | Miscanthus sinensis | Herbaceous    |
| 37   | Noto sacanthini | Natosacantha ihai | 0 (4) | –   | LM  | Turpinia ternata | Woody         |
| 38   | Chrysomelinae | Chrysolina aurichalcea | 9 (21) | –   | ELF | Artemisia indica | Herbaceous    |
| 39   | Chrysomelinae | Chrysolina exanthematica | 5 (15) | –   | ELF | Lycopus lucidus | Herbaceous    |
| 40   | Chrysomelinae | Chrysolina virgata | 5 (20) | –   | ELF | Lycopus lucidus | Herbaceous    |
| 41   | Chrysomelinae | Chrysolena populi | 0 (16) | 0 (7) | ELF | Populus tremula | Woody         |
| 42   | Chrysomelinae | Chrysolena vigintipunctata | 0 (6) | 0 (10) | ELF | Salix triandra | Woody         |
| 43   | Chrysomelinae | Gastrolinea depressa | 0 (10) | 0 (20) | ELF | Juglans mandshurica | Woody         |
| 44   | Chrysomelinae | Gastorniaodes japonicus | 0 (2) | 2 (15) | ELF | Viburnum plicatum | Woody         |
| 45   | Chrysomelinae | Gastrophytum atroranea | 18 (20) | 4 (10) | ELF | Rumex japonicus | Herbaceous    |
| 46   | Chrysomelinae | Gonioctena rubripennis | 0 (9) | 0 (12) | ELF | Wisteria floribunda | Woody         |
| 47   | Chrysomelinae | Lineide aenea | 0 (2) | –   | ELF | Alnus pendula | Woody         |
| 48   | Chrysomelinae | Lineide formosana | 0 (15) | 0 (16) | ELF | Alnus japonica | Woody         |
| 49   | Chrysomelinae | Phaedon brassicae | 12 (12) | 8 (30) | ELF | Raphanus sativus | Woody         |
| 50   | Chrysomelinae | Phala octodecimguttata | 5 (14) | 2 (8) | ELF | Vitex rotundifolia | Woody         |
| 51   | Chrysomelinae | Plagiodera versicolora | 1 (20) | 0 (14) | ELF | Salix chaenomeloides | Woody         |
| 52   | Criocerine | Lilloceris formasana | 8 (11) | –   | ELF | Smilax china | Woody         |
| 53   | Criocerine | Lilloceris mergidera | 1 (3) | –   | ELF | Lilium longiflorum | Herbaceous    |
| 54   | Criocerine | Lilloceris subpolita | 2 (2) | –   | ELF | Smilax china | Woody         |
| 55   | Lemini     | Lema cirscola | 0 (1) | –   | ELF | Commelina communis | Herbaceous    |
| 56   | Lemini     | Lema decempunctata | 0 (1) | –   | ELF | Solanum melongena | Herbaceous    |
| 57   | Lemini     | Lema diversa | 0 (4) | –   | ELF | Commelina communis | Herbaceous    |
| 58   | Lemini     | Oulema tristis | 0 (3) | –   | ELF | Carex sp. | Herbaceous    |
| 59   | Cryptocephalinae | Physosmaragdina nigrifrons | 1 (1) | –   | DF  | Fallopia japonica | Herbaceous    |
| 60   | Cryptocephalinae | Smaragdina nipponensis | 3 (3) | –   | DF  | Celtis sinensis | Woody         |
| 61   | Cryptocephalinae | Smaragdina Semiaurantia | 2 (11) | –   | DF  | Carpesium divaricatum | Herbaceous    |
| 62   | Cryptocephalinae | Cryptocephalus approximatus | 8 (10) | –   | UK   | Wisteria floribunda | Woody         |
### Appendix 1. Continued

#### Leaf beetles

| Code | Subfamily | Tribe | Species | Dropping* | Type† | Host plants |
|------|-----------|-------|---------|-----------|-------|-------------|
|      |           |       |         | Adult | Larva | Species     | Growth form |
| 63   | Cryptocephalini | Cryptocephalus nigrofasciatus | 2 (2) | – | UK | Quercus serrata | Woody |
| 64   | Cryptocephalini | Cryptocephalus perelegans | 3 (9) | – | UK | Rosa multiflora | Woody |
| 65   | Cryptocephalini | Cryptocephalus signaticeps | 1 (2) | – | UK | Rosa multiflora | Woody |
| 66   | Fulcidacini | Chlamisus geniculatus | 1 (1) | – | UK | Quercus serrata | Woody |
| 67   | Fulcidacini | Chlamisus spilotus | 1 (1) | – | UK | Morus australis | Woody |
| 68   | Donaciinae | Donacia lenzi | 0 (8) | – | RF | Nymphaea tetragona | Herbaceous |
| 69   | Donaciini | Donacia ozensis | 0 (3) | – | RF | Nuphar japonica | Herbaceous |
| 70   | Donaciini | Donacia vulgaris | 1 (5) | – | RF | Nuphar japonica | Herbaceous |
| 71   | Haemonini | Donacia japonana | 4 (16) | – | RF | Sparganium sp. | Herbaceous |
| 72   | Eumolpinae | Acrothrinium gaschkevitchii | 7 (10) | – | RF | Vitis ficifolia | Woody |
| 73   | Bromiini | Demotina decorata | 2 (3) | – | UK | Quercus glauca | Woody |
| 74   | Bromiini | Demotina fasciculata | 0 (2) | – | UK | Quercus serrata | Woody |
| 75   | Bromiini | Lypesthes ater | 2 (2) | – | UK | Juglans mandshurica | Woody |
| 76   | Bromiini | Lypesthes fulbus | 1 (2) | – | UK | Cinnamomum yabunikkei | Woody |
| 77   | Bromiini | Lypesthes japonicus | 2 (2) | – | UK | Camellia japonica | Woody |
| 78   | Bromiini | Scelodonta lewisii | 4 (11) | – | UK | Rumex japonicus | Herbaceous |
| 79   | Bromiini | Trichocheysa japonica | 3 (3) | – | UK | Quercus crispa | Woody |
| 80   | Bromiini | Trichocheysa okinawana | 1 (1) | – | UK | Quercus crispa | Woody |
| 81   | Euryopini | Colasposoma auripenne | 2 (2) | – | RF | Ipomoea cairica | Herbaceous |
| 82   | Euryopini | Colasposoma dauricum | 2 (3) | – | RF | Caltystega pubescens | Herbaceous |
| 83   | Typophorini | Basilepta fulvipes | 21 (29) | – | UK | Artemisia indica | Herbaceous |
| 84   | Typophorini | Basilepta rufulicollis | 2 (2) | – | UK | Cerasus × yedoensis | Woody |
| 85   | Typophorini | Pagria sp. | 10 (12) | – | STB | Rhaphiolepis indica | Woody |
| 86   | Galerucinae | Galeruca vicina | 11 (15) | – | ELF | Petasites japonicus | Herbaceous |
## Appendix 1. Continued

| Code | Subfamily | Tribe | Species | Dropping* | Host plants | Growth form |
|------|-----------|-------|---------|-----------|-------------|-------------|
|      |           |       |         | Adult     | Larva       | Type†       | Species             |                       |
| 87   | Galerucini | Galerucella grisescens | 5 (15) | 2 (7) | ELF | Rumex japonicus | Herbaceous |
| 88   | Galerucini | Galerucella nipponensis | 1 (4) | – | ELF | Trapa japonica | Herbaceous |
| 89   | Galerucini | Ophraella communis | 4 (8) | 4 (7) | ELF | Xanthium occidentale | Herbaceous |
| 90   | Galerucini | Pyrrhalta fusciennis | 0 (1) | – | UK | Acer pseudoplatanus | Woody |
| 91   | Galerucini | Pyrrhalta humeralis | 2 (6) | 0 (20) | ELF | Viburnum odoratissimum | Woody |
| 92   | Galerucini | Pyrrhalta lineola | 0 (10) | – | ELF | Salix alba | Woody |
| 93   | Hylaspini | Agelastica coerula | 2 (9) | 0 (20) | ELF | Abies alba | Woody |
| 94   | Hylaspini | Arthrotus niger | 9 (10) | – | ELF | Castanea crenata | Woody |
| 95   | Hylaspini | Gallerucida bifasciata | 5 (5) | 13 (25) | ELF | Fallopia japonica | Herbaceous |
| 96   | Hylaspini | Morphosphaera coerula | 5 (8) | 0 (11) | ELF | Ficus carica | Woody |
| 97   | Luperini | Atrachya menetriesi | 2 (3) | – | ELF | Vicia sp. | Herbaceous |
| 98   | Luperini | Aulacophora bicolor | 6 (8) | – | RF | Diploclados palmatus | Herbaceous |
| 99   | Luperini | Aulacophora indica | 13 (21) | – | RF | Cucumis sativus | Herbaceous |
| 100  | Luperini | Aulacophora loochooensis | 2 (2) | – | RF | Cucurbita maxima | Herbaceous |
| 101  | Luperini | Aulacophora nigripennis | 5 (9) | – | RF | Lagenaria siceraria | Herbaceous |
| 102  | Luperini | Epaenidea elegans | 1 (2) | – | UK | Eleutherodictyon dichotomum | Woody |
| 103  | Luperini | Fleutilia armata | 18 (20) | – | RF | Morus alba | Woody |
| 104  | Luperini | Liroetis coerulea | 1 (10) | – | UK | Quercus acutissima | Woody |
| 105  | Luperini | Monolepta kurosawai | 2 (2) | – | UK | Hovenia dulcis | Woody |
| 106  | Luperini | Monolepta nojiriensis | 6 (12) | – | UK | Styrax japonicus | Woody |
| 107  | Luperini | Monolepta pallidula | 2 (2) | – | UK | Quercus variabilis | Woody |
| 108  | Luperini | Paridea angulicollis | 1 (1) | – | RF | Gynostemma pentaphyllum | Herbaceous |
| 109  | Luperini | Paridea quadriplagiata | 1 (1) | – | RF | Aster yamamotoi | Herbaceous |
| 110  | Lepidoptera | Oides bowringii | 10 (15) | 0 (15) | ELF | Kadsura japonica | Woody |
| 111  | Lamprosomatinae | Oromorphoides cupreatus | 8 (22) | – | UK | Aralia elata | Woody |
| 112  | Lamprosomatinae | Oromorphoides loochooensis | 8 (8) | – | UK | Schefflera heptaphylla | Woody |

*Numbers of individuals dropping from host plants. Values in parentheses indicate the total numbers of individuals investigated in this study.
†Larval feeding type: DF, detritus feeder; ELF, external leaf feeder; LM, leaf miner; RF, root feeder; SEB, seed borer; STB, stem borer; UK, unknown. All adults feed externally on leaves.