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Resistance of Boxwood Varieties to the Boxwood Leafminer, *Monarthropalpus flavus* (Schrank)¹

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Abstract -

A survey of nine cultivars of *Buxus* at the United States National Arboretum revealed significant differences in levels of infestation by the boxwood leafminer, *Monarthropalpus flavus* (Schrank). An analysis of larval survival confirmed high levels of susceptibility in *Buxus sempervirens* 'Myrtifolia' and *Buxus microphylla* 'National' while *B. sempervirens* 'Handsworthiensis' and *B. sempervirens* 'Vardar Valley' exhibited high levels of resistance. Other varieties exhibited intermediate levels of resistance. Field surveys and laboratory studies indicated that female flies deposited eggs in all cultivars even highly resistant ones. This lack of preference suggests that the mechanism of resistance is antibiosis or phenological asynchrony rather than antixenosis.

Index words: Pest management, antibiosis, antixenosis, host selection.

Species used in this study: Boxwoods, *Buxus sempervirens* 'Arborescens' P. Miller; *B.s.* 'Myrtifolia' Gordon, Dermer, and Edmonds; *B.s.* 'Belleville' R. Siebert; *B.s.* 'Suffruticosa' L.; *B.s.* 'Pyramidalis' Simon Louis; *B.s.* 'Handsworthiensis' Fisher; *B.s.* 'Vardar Valley,' D. Wyman; *Buxus microphylla* var. *japonica* 'National' D. Anberg; *B.m.* var. *j.* 'Green Beauty'; and *B. microphylla* var. *japonica* (Muell.) Rehd. and Wils.; Boxwood leafminer, *Monarthropalpus flavus* (Schrank).

Significance to the Nursery Industry

Boxwoods are one of the most common woody plants found in landscapes. The boxwood leafminer, *Monarthropalpus flavus*, is a serious pest of boxwoods in landscapes and nurseries. The production and use of resistant cultivars can provide durable, inexpensive, and environmentally responsible management of insect pests. However, there have been few studies of resistance of boxwoods to leafminer. A field survey of boxwoods revealed high levels of resistance in several varieties. By producing and marketing pest resistant cultivars such as *Buxus sempervirens* 'Handsworthiensis' and *B. sempervirens* 'Vardar Valley' growers should enjoy a marketing advantage to consumers interested in developing sustainable landscapes.

Introduction

Landscape managers and growers need durable, effective, and safe methods for controlling key pests of plants in landscapes and nurseries. While the application of synthetic pesticides can dramatically reduce populations of insect pests and the injury they cause, usually these reductions are temporary. The introduction of biological control agents has proven effective in providing long-term reductions in populations of some pests of landscape plants. Unfortunately, many key pests of ornamental plants lack predators, parasitoids, or pathogens capable of limiting their numbers in most nursery or landscape settings. An alternative approach to reducing problems caused by insect pests is to grow and plant species and cultivars that resist or tolerate colonization and infestation by pests.

Morgan et al. (18), Potter (22), and Raupp et al. (27) discuss advantages and limitations to the more widespread use of resistant plant materials in landscape settings. Advantages include significant reductions in the need for pesticides to control key pests. Limitations include a general paucity of resistant plant materials that are commercially available. However, several recent studies document varying levels of resistance in woody landscape plants to insect and mite pests. These include resistance of azaleas to lace bug (3, 33), buddleia to spider mites (8), maples to gypsy moth (21), cotoneaster to lace bugs (28, 29, 30), euonymus to euonymus scale (12), English ivy to two-spotted spider mite (19), holly to spittle bugs and Florida wax scale (4), juniper to bagworm (13), pine to Nantucket pine tip moth (31), pyracantha to lace bugs (28), oak to orangestriped oakworm (6), linden to gypsy moths and aphids (21, 34), elm to elm leaf beetle (9, 17), crabapple, birch, cherry, linden, and rose to Japanese beetle (26, 25), dogwood to dogwood borer (14), birch to birch learniner, aphids, bronze birch borer and Japanese beetle (14). A list of ornamental plants resistant to insects and diseases has been compiled by Smith-Fiola (32).

A survey of residential landscapes in Maryland revealed that boxwoods comprised about 8.7% of the total woody plants (11). The boxwood leafminer, *Monarthropalpus flavus* (Schrank), was the third most common insect pest requiring control (11). Larvae mine parenchyma tissue of boxwood leaves. Mined leaves are discolored and blistered. This in turn reduces the aesthetic quality of the plant. In heavy infestations, leaves senesce and drop prematurely thus rendering the canopy thin and unsightly. Heavily infested plants are more susceptible to cold injury and winter-kills (2, 15).

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Heavy infestations also attract predatory birds that rip open the galls to eat the larvae (2). Collateral damage from their feeding is often worse than that caused by the leafminers (2, 7). The life history and chemical control of this insect in the middle-Atlantic region of the United States have been discussed in greater detail elsewhere (7).

The objective of this study was to investigate the relative susceptibility of boxwood cultivars by evaluating the survival of immature leafminers and ovipostion behavior of adults. We conducted a survey of boxwoods in a common garden at the United States National Arboretum in Washington, DC, to determine levels of attack by adult flies, abundance of larvae, and survival of immature stages. We also investigated the egg-laying behavior of adult flies in the greenhouse to determine if some cultivars were more heavily attacked than others.

Materials and Methods

Common garden survey. In April 1997, nine cultivars of boxwoods were surveyed at the United States National Arboretum in Washington, DC. All were part of a boxwood collection in a common garden. The garden contains more than one hundred individual plants many of which were infested with boxwood leafminer. Boxwoods included in the survey were exposed to M. flavus adults emerging from infested plants in the garden. All plants were established for more than five years and ranged in height from about 1 to 4 m (3.2-13 ft). The following cultivars were sampled: Buxus sempervirens 'Arborescens' P. Miller; B.s. 'Myrtifolia' Gordon, Dermer, and Edmonds; B.s. 'Belleville' R. Siebert; B.s. 'Suffruticosa' L.; B.s. 'Pyramidalis' Simon Louis; B.s. 'Handsworthiensis' Fisher; B.s. 'Vardar Valley,' D. Wyman; Buxus microphylla var. japonica 'National' D. Anberg; and B.m. var. japonica (Muell.) Rehd. and Wils. Three plants of each cultivar were sampled. To assess leafminer attack rates, larval abundance, and immature survival, five branches on each plant were randomly selected and sampled. To help reduce variation associated with differences in leaf age, a leaf of the same plastochron index (same position from the terminal) was selected from each branch.

Two attributes of boxwood leafminer activity and abundance were recorded from each leaf. The deposition of eggs into boxwood leaves forms a distinct scar on the leaf surface (7). By recording the number of scars we estimated the number times females deposited eggs (7). Excised leaves were dissected and the number of larvae recorded. These counts provided an estimate of larval abundance. Survival of immature stages was estimated by dividing the number of larvae found in a leaf by the number of oviposition scars on the leaf surface. Variances among treatments (cultivars) were large and heteroscedastic for all three dependent variables. Homogeneity of variance could not be achieved through transformation of the data. Therefore, oviposition, larval abundance, and juvenile survival were compared among cultivars with a Kruskal-Wallis nonparametric analysis of variance (35). Differences among treatments were resolved following the Kruskal-Wallis analysis with a Nemenyi test (35).

Ovipositional preference in the greenhouse. To measure the ability of adults to discriminate among boxwood cultivars for egg-laying, and thereby test for a nonpreference (antixenosis) mode of resistance, flies were confined in cages and allowed to oviposit on four different cultivars in both

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choice and no-choice experiments. These experiments were conducted in the greenhouse at the University of Maryland, College Park, MD. The cultivars chosen for this study were *B.s.* 'Arborescens', *B.s.* 'Suffruticosa', *B.s.* 'Vardar Valley' and *B.m.* var. *japonica* 'Green Beauty'. We selected *B.s.* 'Vardar Valley' and *B.s.* 'Arborescens' to permit a direct comparison of one cultivar that was heavily infested by leafminers in the common garden survey described above to another that was not. The remaining varieties were selected on the bases of popularity in the market, as described by boxwood growers, and availability of small plants that could be easily used in the study.

To evaluate the oviposition behavior of adult flies, two sets of experiments were conducted. The first investigated egg-laying behavior of adult flies when no choice of cultivars was possible. Four specimens of each cultivar in #1 containers were placed in 1 m \times 1 m \times 1 m (3.3 ft \times 3.3 ft \times 3.3 ft) box cages covered with fine mesh. Prior to the experiments several 0.15 m (0.5 ft) branches containing mature pupae were collected from a single B.s. 'Arborescens' and placed in water picks. One branch infested with pupae was placed in the center of each cage to provide a source of adult flies. Infested branches were randomly assigned to each cage and although direct counts of pupae could not be made we observed that approximately equal numbers of flies emerged from each branch. Flies were allowed to emerge, mate, and oviposit for one week. All leaves on each plant were examined and the number of oviposition scars counted. This experiment was replicated 4 times with each of the four cultivars evaluated.

To compare oviposition behavior of flies when a choice of cultivars was possible, treatments were established that contained one representative of each of the four cultivars. Their placement in the cages was varied to account for possible heliotropism by ordering the location of each plant to the cardinal points within the cage. All plants were of approximately the same size in #1 containers. Flies were introduced as in the previous study and allowed to emerge and oviposit for one week. This experiment was replicated three times.

Oviposition behavior on different cultivars was compared in the choice and no-choice experiments with an analysis of variance (35). The cardinal locations of plants within cages were similarly analyzed with an analysis of variance to determine if there was any effect of heliotopism of host selection by adults (35).

Results and Discussion

Common garden survey. The survey of boxwood species and cultivars in a common garden revealed highly significant differences in the ovipostion behavior of adult flies (χ^2 = 21.80, P < 0.005). The rank order of cultivars as oviposition sites for the leafminer from most heavily attacked to least was *B.s.* 'Myrtifolia', *B.s.* 'Belleville', *B.m.* var. *japonica* 'National', *B.s.* 'Arborescens', *B.s.* 'Pyramidalis', *B.s.* 'Suffruticosa', *B.s.* 'Handsworthiensis', *B.s.* 'Vardar Valley,' and *B.m.* var. *japonica* (Fig. 1).

The number of larvae found in leaves also differed significantly among cultivars ($\chi^2 = 25.03$, P < 0.002). The rank order of cultivars with respect to larval abundance from most to least was: *B.s.* 'Myrtifolia', *B.m.* var. *japonica* 'National', *B.s.* 'Belleville', *B.s.* 'Arborescens', *B.m.* var. *japonica*, *B.s.* 'Suffruticosa', *B.s.* 'Pyramidalis', *B.s.* 'Handsworthiensis', and *B.s.* 'Vardar Valley' (Fig. 1). *Buxus sempervirens*



Fig. 1. Number of ovipostion scars and boxwood leafminer larvae associated with leaves of nine boxwood cultivars at the United States National Arboretum. Abbreviations are as follows. Myr. = Buxus sempervirens 'Myrtifolia', Bel. = B.s. 'Belleville', Arb. = B.s. 'Arborescens', Suf. = B.s. 'Suffruticosa', Pyr. = B.s. 'Pyramidalis', Var. = B.s. 'Vardar Valley', Han. = B.s. 'Handsworthiensis', Jap. = Buxus microphylla var. japonica, Nat. = B. microphylla var. japonica 'National'. Bars represent means. Vertical lines represent standard errors. Means that share the same letter do not differ significantly by a Nemenyi test (P = 0.05). Capital letters are for comparisons of oviposition scars, lower case for comparisons of larvae.

'Suffruticosa' and *B.s.* 'Pyramidalis' supported less than one larva per leaf. No living larvae were found in the leaves of *B.s.* 'Handsworthiensis' or *B.s.* 'Varder Valley' (Fig. 1).

Differences in host selection by females could explain, at least in part, lower abundance of larvae where oviposition scars were few. However, females utilized all varieties for oviposition. The use all cultivars as oviposition substrates indicates that nonpreference or antixenosis was not the primary mode of resistance to the leafminer (16). Analysis of survival rates indicated highly significant differences in the ability of eggs and larvae to survive in different cultivars ($\chi^2 = 24.26$, P < 0.002). Survivorship was highest in the *B. microphylla* varieties *japonica* and *japonica* 'National' and lowest in *B.s.* 'Handsworthiensis' and *B.s.* 'Varder Valley' (Fig. 2). The low rates of immature survival in several cultivars suggests that antibiosis rather than antixenosis is a more likely mechanism underlying observed differences in larval abundance (20).

Ovipositional preference in the greenhouse. When four boxwood cultivars were provided as oviposition substrates for flies in a no-choice setting, that is when cages contained four specimens of the same cultivar, there was no significant difference in the number of oviposition scars among the four varieties examined ($F_{3,44} = 2.05$, P < 0.121). The same result was observed when flies were given a choice of cultivars by placing four cultivars in the same cage. No significant difference in the number of oviposition scars was observed

among cultivars ($F_{3,8} = 0.64$, P < 0.61). Within each cage, the position of the plant with respect to cardinal orientation had no effect on the number of eggs laid in both studies ($F_{3,59} = 2.24$, P < 0.093).

Early reports of resistance in *Buxus* to attack by the *M*. *flavus* indicated that *B. sempervirens* L. was a highly susceptible host for the leafminer but that several other species and cultivars were attacked (1). One species first thought to be resistant to leafminer attack, *B. sempervirens* 'Bullata' G. Kirchner, later proved to be susceptible (5). Brewer and Skuhravy (5) investigated susceptibility of three varieties of *Buxus* including *B. sempervirens* 'Bullata' to the leafminer. They confirmed the susceptibility of *B. sempervirens* 'Bullata' while *B. sempervirens* 'Nana' V. Veillard (= 'Suffruticosa') and *B. sempervirens* were less so. Unfortunately, winter-kill in several replicates caused some results of these studies to be equivocal (5).

Results of our study provide evidence for high levels of resistance in at least two varieties of boxwood, *B.s.* 'Handsworthiensis' and *B.s.* 'Varder Valley' where extraordinarily low densities of larvae were found. Survival rates of immature stages in these cultivars were similarly very low. *Buxus sempervirens* 'Suffruticosa' and *B.s.* 'Pyramidalis' also supported very low densities of larvae in plants surveyed in the common garden. One possible explanation for lower densities of leafminers in these cultivars could be an avoidance or lack of preference by ovipositing flies. This form of resistance, first termed nonpreference by Painter (20) and later



Fig. 2. Survivorship of boxwood leafminer larvae in leaves of nine boxwood cultivars at the United States National Arboretum. Abbreviations are as follows. Myr. = Buxus sempervirens 'Myrtifolia', Bel. = B.s. 'Belleville', Arb. = B.s. 'Arborescens', Suf. = B.s. 'Suffruticosa', Pyr. = B.s. 'Pyramidalis', Var. = B.s. 'Vardar Valley', Han. = B.s. 'Handsworthiensis', Jap. = Buxus microphylla var. japonica, Nat. = B. microphylla var. japonica 'National'. Bars represent means. Vertical lines represent standard errors. Means that share the same letter do not differ significantly by a Nemenyi test (P = 0.05).

called antixenosis by Kogan and Ortman (16), is unlikely to explain the patterns of relative resistance observed in the field. All cultivars were attacked and used as oviposition substrates. The host choice experiment conducted in the greenhouse indicated that female flies fail to discriminate among varieties that favor larval survival such as *B.s.* 'Suffruticosa' and varieties where larval survival is low such as *B.s.* 'Vardar Valley'. These two pieces of evidence make antixenosis an unlikely mechanism for explaining differences in the abundance of leafminers in leaves of different cultivars.

A more likely explanation for the observed resistance is that several of the boxwoods tested are either nutritionally inferior or contain allelochemicals suppressive to the growth and survival of leafminer eggs or larvae. This form of resistance has been termed antibiosis (20). A final possibility, one treated in a general sense by Painter (20) and specifically in the case of boxwoods by Brewer and Skuhravy (5), is that phenological asynchrony between the ovipositing leafminers and the availability of tender host leaves may reduce the success of the fly in colonizing some varieties. The importance of synchrony between leafminer emergence, egg deposition, and the availability of young, tender, host leaves has been well documented in other associations involving dipterous leafminers and their broadleaved evergreen hosts by Potter and Kimmerer (23) and Potter and Redmond (24).

In summary, boxwoods in a common garden demonstrated a great range of susceptibility to a key insect pest, the boxwood leafminer. Some cultivars such as B.s. 'Myrtifolia', B.m. var. japonica 'National', B.s. 'Belleville', and B.s. 'Arborescens' were heavily attacked and readily supported survival and development of immature stages of the leafminer. Other cultivars such as B.s. 'Handsworthiensis' and B.s. 'Varder Valley' were attacked by ovipositing flies but did not support the survival of immature stages of the leafminer. By producing and marketing resistant cultivars such as B.s. 'Handsworthiensis' and B.s. 'Varder Valley', growers could realize a significant marketing advantage. Some producers and consumers may not want to use insecticides to manage the boxwood leafminer. Moreover, recent legislative action has removed some insecticides previously used to manage this pest. Although highly efficacious insecticides are still available (7), landscapers and consumers who plant resistant cultivars should have to treat less frequently or, perhaps, not at all for the leafminer. Landscape managers and owners who have highly susceptible cultivars will have to manage these plants more intensively. This may include more frequent inspections for pest activity and intervention to reduce leafminer populations. Future research will continue to identify boxwood cultivars resistant to this and other arthropod pests and to elucidate the mechanisms of resistance.

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