

Gradients in Susceptibility and Resistance Mechanisms of *Buddleia* L. Taxa to the Two-spotted Spider Mite (*Tetranychus urticae* Koch)

Jeffrey H. Gillman,¹ Michael A. Dirr,² and S. Kristine Braman³

Department of Horticulture, The University of Georgia, Athens, GA 30602

ADDITIONAL INDEX WORDS. butterfly bush, host plant resistance, insect resistance, trichome, pubescence

ABSTRACT. *Buddleia* taxa were assessed for two-spotted spider mite (*Tetranychus urticae* Koch) resistance using a leaf disk bioassay, a novel shell vial bioassay and a field trial. Leaf pubescence and chemistry were examined for their role in two-spotted spider mite resistance. Results from bioassays and field sampling identified highly resistant taxa including *B. fallowiana* Balif. 'Alba' and *B. davidii* × *B. fallowiana* Franch. 'Cornwall Blue' as well as susceptible taxa including *B. davidii* Franch. 'African Queen' and *B. lindleyana* Fort. ex Lindl. 'Gloster'. The shell vial bioassay was an accurate predictor of field resistance to spider mite. Leaf pubescence was quantified by calculating the collective length of trichome branches per square millimeter of leaf surface area [effective branch length (EBL)]. EBL values ranged from 39 to 162 mm·mm⁻² of leaf surface area among *Buddleia* taxa. Resistance was positively correlated with increased pubescence. Removal of pubescence by peeling resulted in increased oviposition of two-spotted spider mites. Exposing female two-spotted spider mites to a methylene chloride extract of *B. davidii* × *B. fallowiana* 'Cornwall Blue' using a modified shell vial bioassay resulted in reduced oviposition and a methylene chloride extract of *B. davidii* 'African Queen' resulted in no difference in oviposition when compared with a control. While pubescence is the best indicator of resistance to the two-spotted spider mite in *Buddleia* taxa, it is possible that defensive compounds are involved.

Buddleia taxa, butterfly bushes, are popular ornamental shrubs grown for their large fragrant inflorescences that attract nectar feeding butterflies to a garden. The most damaging pest of *Buddleia* species is the two-spotted spider mite, *Tetranychus urticae* Koch, (Maunder, 1987) whose feeding causes stippling, chlorosis, and leaf abscission (Metcalf and Metcalf, 1993), and results in plants that are aesthetically displeasing and difficult to market.

The two-spotted spider mite is one of the most damaging arthropods to nursery and greenhouse crops in the southeast (Hudson et al., 1996; Baker, 1980) and requires frequent pesticide inputs for control (Hudson et al., 1996). Many crops, both horticultural and agronomic, have been examined for resistance to the two-spotted spider mite including *Gossypium hirsutum* L. (Schuster et al., 1972), *Cucumis sativus* L. (Knipping et al., 1975), *Rubus idaeus* L. (Wilde et al., 1991), and *Fragaria* sp. L. (Ferrer et al., 1993). Woody landscape ornamentals, such as the shrubby *Buddleia* taxa, have not been evaluated for their resistance to the two-spotted spider mite. Selection of resistant taxa would prove economically and aesthetically beneficial to growers and gardeners.

The ability of *Buddleia* species to resist infestation by arthropod pests has been noted in various literature sources (Dirr, 1998; Owen and Whiteway, 1980). This resistance is thought to be associated with plant chemistry (Owen and Whiteway, 1980), which is supported by research showing that, at least in the case of *B. lindleyana* Fort. ex Lindl., compounds toxic to arthropods are present (Chin et al., 1947). Many *Buddleia* taxa, including *B. davidii*, contain novel sesquiterpenes and alkaloids (Roder et al., 1985; Stermitz and Harris, 1985; Yoshida et al., 1978). The ability of these compounds in *Buddleia*

taxa to reduce arthropod feeding, however, is unknown although extracts of *B. madagascariensis* Lam. (Shoeb et al., 1991) have been shown to be toxic to mollusks. Glandular trichomes that may contain defensive compounds are present on the leaves of *Buddleia* species (Rogers, 1986).

A second mechanism that may affect arthropod herbivory in *Buddleia* taxa is the presence of highly branched, nonglandular trichomes (Rogers, 1986). Although the ability of these trichomes to prevent herbivory is not well characterized, there is evidence that they are defensive structures that limit arthropod feeding. *Cleopus japonicus* Wingelmuller, a weevil that is host specific to *Buddleia*, avoids the trichomitous undersurface of *B. davidii* leaves (Zhang et al., 1993) and feeds on the upper surface. This feeding behavior indicates that mechanisms present on the underside of *Buddleia* leaves, most probably trichomes, are affecting the weevil's ability to feed successfully on this portion of the leaf.

Standard assays for assessing the relative susceptibility of particular plants to the two-spotted spider mite frequently consist of leaf disk bioassays (de Ponti, 1977a; Ferrer et al., 1993; Wilde et al., 1991). Leaf disk bioassays rapidly provide information regarding the effects of cultivar, pesticides, and even nutrient regimes, on small arthropods such as spider mites. These assays, however, are confounded when nonpreference effects cause spider mites to exit leaf disks of plants that they normally infest despite the presence of viscous materials surrounding, and intended to keep mites on, the experimental surface (Aina et al., 1972; Knipping et al., 1975; Mansour and Karchi, 1990; Wilde et al., 1991). The exit of spider mites from leaf disks creates a confounding effect when counting the number of eggs laid by female mites over time, the criterion most commonly used for assessing resistance (de Ponti, 1977a; Ferrer et al., 1993; Wilde et al., 1991). Although this problem has been rectified by certain pesticide bioassays in which compounds are applied directly to the surface of a sealed container holding the spider mites (Kabir et al., 1996), these assays are not practical for testing leaf tissue or for assessing any variable except mite mortality. A second confounding factor associated with standard leaf disk bioassays is the orientation of leaf disks in a fashion that is unfamiliar to spider mites who commonly feed in an inverted position on the underside of leaves. Most leaf disk

Received for publication 27 Apr. 1998. Accepted for publication 16 Nov. 1998. We thank Hazel Wetzstein for technical assistance. Use of trade names does not imply endorsement of products named or criticism of ones not mentioned. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

¹Assistant professor, Department of Horticultural Science, University of Minnesota, St. Paul, MN 55108.

²Professor, Department of Horticulture, University of Georgia, Athens, GA 30602.

³Associate professor, Department of Entomology, Georgia Experiment Station, Griffin, GA 30223.

bioassays allow mites to feed on disks which have been placed upside-down in a petri dish, thus forcing the mite to feed in an upright orientation. The problem of incorrect orientation of leaf disks has been addressed by researchers, working with the broad mite, by placing leaf disks onto solidifying agar and then inverting the entire plate (Herron et al., 1996). This system controls orientation, but still offers mites the opportunity to exit the experimental surface onto a medium which could cause mortality.

The objective of the current research was to test the hypothesis that there are differences in two-spotted spider mite resistance among *Buddleia* taxa and, having demonstrated resistance, to determine the effects of *Buddleia* leaf pubescence and leaf surface compounds on two-spotted spider mite resistance.

Materials and Methods

LEVELS OF RESISTANCE AMONG *Buddleia* TAXA. Two-spotted spi-

Table 1. *Buddleia* taxa susceptibility to the two-spotted spider mite as measured by mites remaining on leaf disks and number of eggs laid by 4 female spider mites on leaf disks after 48 and 96 h (error df = 148; 48-h mites F = 2.58, $P < 0.01$; 48-h eggs F = 2.28, $P < 0.01$; 96-h mites F = 1.85, $P < 0.01$; 96-h eggs F = 2.68, $P < 0.01$).

Species or cultivar	Time (h)			
	48		96	
	Mites/disk	Eggs/disk	Mites/disk	Eggs/disk
<i>B. lindleyana</i> Fort. Ex Lindl Gloster	2.0 abc ^z	7.6 ab	2.2 abcd	8.2 abc
<i>B. davidii</i> Franch. African Queen	2.4 ab	3.4 abcd	2.4 a	7.0 a
<i>B. lindleyana</i> Mayflower	1.4 abcde	5.6 abcde	1.0 abcdef	6.8 abcdef
<i>B. hemsleyana</i> Hemsl.	1.8 abcde	3.6 abc	1.0 abcdef	6.4 ab
<i>B. davidii</i> White Profusion	1.4 bcdef	5.4 abc	0.6 defg	6.2 abcd
<i>B. colvillei</i> Hook. f & Thoms.	2.4 ab	5.2 a	1.4 abcdef	5.6 ab
<i>B. globosa</i> Hope.	2.2 ab	5.0 ab	1.4 abcdef	4.8 abcd
<i>B. japonica</i> Hemsl.	1.0 cdefg	2.6 abcd	1.2 abcdef	4.2 abcde
<i>B. davidii</i> White Harlequin	2.0 abc	2.6 abcde	1.6 abcd	3.8 abcd
<i>B. salvifolia</i> L.	1.6 abcde	3.2 abcd	1.6 abcdef	3.2 abcdefg
<i>B. davidii</i> var. <i>nanhoensis</i> Petite Plum	2.0 abcd	2.4 abcdef	0.4 gf	3.0 bcdefghi
<i>B. asiatica</i> Lour.	2.2 ab	2.0 abcde	2.0 abc	2.2 abcdefgh
<i>B. davidii</i> Miss Ellen	3.2 a	1.6 abcdef	1.8 abcd	2.2 bcdefghi
<i>B. nivea</i> Duthie.	2.0 abc	1.4 abcdef	1.8 abcd	1.8 bcdefghi
<i>B. davidii</i> Fascination	1.8 abcd	1.0 bcdef	1.8 abcd	1.8 cdefghi
<i>B. davidii</i> Summer Beauty	0.6 efg	0.6 def	0.8 abcdef	1.6 bcdefghi
<i>B. macrostachya</i> Benth. in Wall.	2.2 ab	0.6 def	2.2 ab	1.4 cdefghi
<i>B. davidii</i> Mary's White	1.2 bcdef	0.8 cdef	0.8 cdefg	1.4 cdefghi
<i>B. weyeriana</i> Weyer. Honeycomb	1.2 bcdef	0.6 def	0.8 cdefg	1.4 cdefghi
<i>B. davidii</i> Bonnie	1.2 abcde	0.2 ef	1.0 abcdef	1.2 defghi
<i>B. davidii</i> Dubonnet	1.6 abcde	0.4 def	0.8 cdefg	1.2 cdefghi
<i>B. davidii</i> Black Knight	1.0 cdefg	0.6 def	0.8 cdefg	1.0 efghi
<i>B. crispa</i> Benth.	2.0 abcd	0.4 def	1.6 abcde	0.8 defghi
<i>B. davidii</i> Pink Charmer	1.2 abcde	0.2 ef	1.4 abcde	0.6 efghi
<i>B. davidii</i> Pink Charming	1.0 cdefg	0.8 cdef	1.0 bcdef	0.6 fghi
<i>B. davidii</i> Border Beauty	0.8 defg	0.2 ef	1.0 bcdef	0.4 ghi
<i>B. davidii</i> Empire Blue	0.6 efg	0.2 ef	0.8 cdefg	0.4 fghi
<i>B. fallowiana</i> Balf. Alba	0.8 defg	0.6 def	0.6 efg	0.4 fghi
<i>B. davidii</i> x <i>B. fallowiana</i> Lochinch	0.8 efg	0.0 f	0.4 fg	0.4 ghi
<i>B. davidii</i> Pink Delight	1.2 bcdef	0.2 ef	1.0 abcdef	0.2 hi
<i>B. davidii</i> Royal Red	0.4 fg	0.2 ef	0.4 fg	0.2 hi
<i>B. davidii</i> Nanho White	1.4 abcde	0.0 f	1.4 abcde	0.0 i
<i>B. davidii</i> Burgundy	1.8 abcde	0.0 f	1.2 abcdef	0.0 i
<i>B. davidii</i> Charming	1.4 bcdef	0.0 f	1.0 defg	0.0 i
<i>B. weyeriana</i> Sungold	0.2 g	0.0 f	0.6 efg	0.0 i
<i>B. davidii</i> x <i>B. fallowiana</i> Cornwall Blue	0.8 defg	0.0 f	0.4 fg	0.0 i
<i>B. davidii</i> Ile de France	0.2 g	0.0 f	0.0 g	0.0 i

^zMeans in a column followed by the same letter are not significantly different ($\alpha = 0.05$) using Fisher's protected LSD test.

Table 2. Eggs laid by five female two-spotted spider mites on nine *Buddleia* taxa after 96 h in shell vial (error df = 168, F = 15.49, P < 0.01).

Species or cultivar	Eggs laid (no.)
<i>B. davidii</i> African Queen	145.5 a ²
<i>B. lindleyana</i> Gloster	74.5 b
<i>B. davidii</i> Ile de France	59.8 b
<i>B. weyeriana</i> Sungold	54.3 b
<i>B. davidii</i> Black Knight	47.0 bc
<i>B. davidii</i> Miss Ellen	32.5 cd
<i>B. davidii</i> Nanho White	28.1 cd
<i>B. davidii</i> × <i>B. fallowiana</i> Cornwall Blue	20.7 de
<i>B. fallowiana</i> Alba	9.7 e

²Means followed by the same letter are not significantly different ($\alpha = 0.05$) using Fisher's protected LSD test.

taxa, were placed top side down onto 9-cm circles of #1 filter paper (Whatman, Kent, England) that had been placed into glass petri dishes (9 cm in diameter) and moistened with 2 mL of distilled water. Leaf disks were ringed with petroleum jelly and four adult female two-spotted spider mites were placed onto each disk. Mites came from a culture raised on bean plants (*Phaseolus vulgaris* L. 'Kentucky Wonder' and 'White Half Runner'). Petri dishes were sealed with parafilm to reduce desiccation of leaf disks and were subsequently placed into an incubator at 25 ± 1 °C with a photoperiod of 12 h light ($24 \pm 3 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$): 12 h dark. Leaf disks were evaluated 48 and 96 h after the introduction of mites, at which time the number of mites remaining and number of eggs laid on each disk were recorded. Data were transformed [\sqrt{x}] and subjected to analysis of variance (version 6.10; SAS, Cary, N.C.). Mean separation was accomplished using Fisher's protected LSD test.

SHELL VIAL BIOASSAY. Nine *Buddleia* taxa (Table 2), selected on the basis of results from the leaf disk bioassay, were tested for two-spotted spider mite resistance using a shell vial bioassay. These taxa were grown under greenhouse conditions and were watered and fertilized as needed with 200 ppm N as Peters 20–10–20 (20N–4.0P–17K) (Scotts-Sierra Hort. Prod. Co. Marysville, Ohio). Leaves were selected randomly from populations of not less than five individual *Buddleia* from each taxon. Five-dram glass shell vials (27.25 mm in diameter × 55 mm in length) with polyethylene two piece closures (Fisher Scientific, Pittsburgh, Pa.) were modified by removing the top from the cap of the two-piece closure and making 16 evenly spaced vertical cuts along the upper portion of the cap that surrounded the orifice revealed by removing the top (Fig. 1). Five female two-spotted spider mites, collected from the same culture as that used for the preliminary leaf disk bioassay, were placed into the glass portion of the shell vial using a horse-hair brush. A young fully expanded leaf from the *Buddleia* taxon was placed bottom side down over the mouth of the shell vial, exposing the central basal section of the leaf to the mites. A piece of damp filter paper ($\approx 5 \times 2$ cm) was then folded into the cap and the cap was placed over the leaf and the bottom portion of the shell vial. Parafilm was wrapped around the cap to ensure a firm closure between the cap and the shell vial. Shell vials were placed into an illuminated incubator at 25 ± 1 °C with a photoperiod of 12 h light ($24 \pm 3 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)/12 h dark. After 96 h, the number of eggs laid per shell vial was determined by examining with a dissecting scope (20×) the portion of the leaf that had been exposed to the spider mites. Four blocks which were separated by time and which included five shell vials each were run for all *Buddleia* taxa. Data were transformed [\sqrt{x}] and subjected to analysis of variance (version 6.10; SAS). Mean separation was accomplished using Fisher's protected LSD test.

FIELD TRIAL. Eleven different *Buddleia* taxa, including all of the

taxa evaluated using the shell vial bioassay as well as *B. davidii* Franch. 'Pink Delight' and 'Bonnie', were planted, with two replications each, into four randomized blocks at the University of Georgia Horticulture Farm in Oconee County, Ga., in July 1996. Plants that died over the ensuing winter were replanted in March, 1997. Plants were fertilized with 10–10–10 (10N–4.0P–8.5K) (Gold Kist, Inc., Atlanta, Ga.) and watered as needed using drip irrigation. Naturally occurring mite and beneficial arthropod populations were sampled from all of the replications on 8 May and every 10 d (± 1 d) from 14 May through 5 Sept. Sampling was accomplished by counting the number of two-spotted spider mites and spider mite eggs on a 6-cm² section of the basal portion of five mature leaves collected randomly from each of the shrubs, except on 8 May when only three leaves per shrub were sampled. Leaves were collected from the second to fourth fully expanded leaves below shoot termini. Data were transformed [\sqrt{x}] and subjected to analysis of variance (version 6.10; SAS). Mean separa-

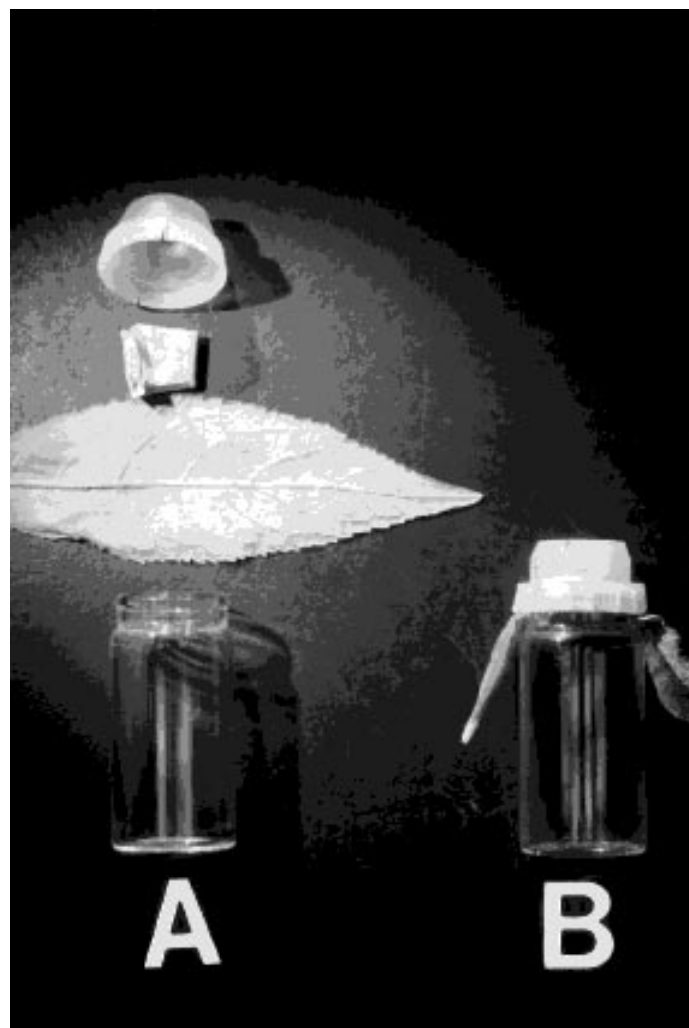


Fig. 1. (A) Components of apparatus used for shell vial bioassays constructed from a 5-dram shell vial with corresponding two piece polyethylene closure: The cap from the polyethylene two piece closure is modified by removing the top and cutting flanges along the remaining orifice. Moist filter paper (5 × 2 cm) is then folded into the cap to provide moisture for the experimental leaf which is placed bottom side down over the 5-dram glass shell vial that contains five female two-spotted spider mites. The cap is then snapped over the leaf and the opening of the glass vial and the apparatus is sealed with parafilm. (B) Assembled apparatus for shell vial bioassay. The modified shell vial bioassay is the same as above except that a bean leaf is used as the food source and a treated tissue inundated with holes separates the leaf from the mites.

Table 3. Two-spotted spider mite motile stages (adults and nymphs) and eggs found per 6 cm² of leaf area on *Buddleia* taxa from 8 May through 13 June field trials (error df = 2148: average mites F = 48.16, *P* < 0.01, average eggs F = 30.42, *P* < 0.01).

Cultivar	8 May		14 May		25 May		3 June		13 June		Avg	
	Mites	Eggs	Mites	Eggs	Mites	Eggs	Mites	Eggs	Mites	Eggs	Mites	Eggs
	Number											
Gloster ^y	4.0 a ^z	63.5 a	4.6 a	25.9 a	5.7 a	13.7 bc	7.9 a	12.6 ab	1.0 cd	3.2 ab	4.8 a	19.9 a
Pink Delight ^x	3.1 ab	14.0 b	3.2 ab	0.9 b	4.1 ab	8.1 cd	4.0 bcd	4.0 bc	1.7 ab	1.5 ab	3.3 b	5.5 c
African Queen ^x	3.0 ab	21.1 b	1.2 cd	5.9 b	5.5 a	22.2 ab	4.4 bc	18.1 a	0.9 bcd	6.9 a	3.2 b	14.8 b
Bonnie ^x	0.3 d	0.2 b	1.8 bc	2.8 b	1.9 cd	6.3 cd	6.0 ab	19.7 a	2.6 a	6.7 a	2.6 cd	8.0 c
Sungold ^w	1.3 cd	1.0 b	2.1 bc	1.4 b	3.1 bc	26.2 a	3.6 bcd	16.9 a	1.0 cd	3.8 ab	2.4 bc	12.3 b
Ile de France ^x	2.6 abc	2.5 b	1.3 cd	1.2 b	2.5 bcd	3.9 d	1.3 cde	2.6 bc	0.2 cd	0.1 b	1.6 cde	2.3 cd
Nanho White ^x	1.6 bcd	0.7 b	1.9 bc	1.7 b	1.9 cd	1.6 d	0.8 de	1.2 c	0.8 cd	0.4 b	1.5 def	1.3 de
Black Knight ^x	0.3 d	0.0 b	0.9 cd	0.7 b	2.2 cd	3.8 d	2.2 cde	3.8 bc	0.4 cd	0.5 b	1.4 def	2.2 cd
Cornwall Blue ^v	1.2 cd	0.9 b	1.9 bc	0.7 b	1.4 de	5.1 cd	0.8 de	1.2 c	0.1 cd	0.1 b	1.0 ef	1.8 d
Miss Ellen ^x	0.2 d	0.0 b	0.6 cd	0.3 b	1.1 de	2.6 d	1.1 de	1.5 c	0.2 cd	0.5 b	0.7 f	1.2 de
Alba ^u	0.0 d	0.0 b	0.0 d	0.0 b	0.0 e	0.0 d	0.0 e	0.0 c	0.0 d	0.0 b	0.0 g	0.0 e

^zMeans in a column followed by the same letter are not significantly different ($\alpha = 0.05$) using Fisher's protected LSD test.

^y*Buddleia lindleyana*.

^x*Buddleia davidii*.

^w*Buddleia weyeriana*.

^v*Buddleia davidii* × *B. fallowiana*.

^u*Buddleia fallowiana*.

tion was accomplished using Fisher's protected LSD test.

QUANTIFICATION OF PUBESCENCE. Five fully expanded young leaves were selected randomly from populations of at least five *Buddleia* plants of the same taxa as investigated in the shell vial bioassay. Plants were grown as described for the shell vial bioassay. Pubescence was quantified by removing epidermal peels from the basal central portion of the underside of each leaf and examining them, using a compound microscope (100×) with a micrometer, to determine the length of branches originating from dendritic trichomes as well as the number of trichome branch points per unit area. Sixteen branches were measured and four observations on the number of branches per 0.13 mm² were tabulated per leaf. Data were then converted to millimeters of trichome branches per square millimeter of leaf surface area by multiplying: (average length of branches) × (average number of branch points per square millimeter) × [number of branches originating from each branch point (four, except for *B. lindleyana*, which has six)]. The result was termed Effective Branch Length (EBL). Branch lengths and number of branch points per square millimeter were confirmed using electron microscopy. EBL was then correlated with resistance results from the previously mentioned shell vial bioassay using regression analysis (version 6.10; SAS). Significant differences in branch length and number of branches per square millimeter were determined using Fisher's protected LSD test (version 6.10; SAS).

REMOVAL OF TRICHOMES AND BIOASSAY OF MITES ON TRICHOMELESS PLANTS. The leaves of four *Buddleia* taxa, representing a range of resistance from most to least susceptible, including *B. davidii* 'African Queen', 'Black Knight', and 'Miss Ellen' and *B. davidii* × *B. fallowiana* Balif. 'Cornwall Blue', were used to determine whether pubescence has an effect on resistance to two-spotted spider mite. A novel bioassay was developed to test the effect of pubescence on spider mite fecundity. Pubescence was removed from young fully expanded leaves of *Buddleia* taxa by applying Freeman's facial peel (Freeman's Cosmetic Co., Beverly Hills, Calif.) to the undersurface of leaves and allowing the peel to dry for ≈1 h. before being removed to expose the epidermal layer of the leaves. Treatments included complete pubescence removal and no pubescence removal. Five female two-spotted spider mites were allowed to oviposit for 96 h on experimental leaves using a shell

vial bioassay as previously described. Two blocks, each including five experimental shell vials of each treatment and taxon, were run. Differences in two-spotted spider mite egg deposition between treatments within each taxon were evaluated using Student's *t* test (version 6.10; SAS).

EFFECT OF LEAF SURFACE COMPOUNDS ON FECUNDITY. The ability of leaf surface compounds present in *Buddleia* taxa to reduce two-spotted spider mite egg laying was assessed via the following bioassay, termed a modified shell vial bioassay. Compounds soluble in methylene chloride were extracted from the leaves of *B. davidii* 'African Queen' and *B. davidii* × *B. fallowiana* 'Cornwall Blue', by dipping fully expanded young leaves (160 ± 5 cm²) into 10 mL of methylene chloride for ten seconds. This extract (0.56 ml) was applied to 9-cm² sections of tissue (Kimwipes; Kimberly-Clark, Roswell, Ga.) at the same concentration as it had been on the surface of the leaf. Each tissue was punctured at a density of 22.5 holes/cm² by pushing a hypodermic needle 1 mm in diameter (19 gauge) through a cardboard template before the application of methylene chloride and extract. Holes allowed mites to cross from the glass shell vial to a food source while forcing them to come in contact with the treated tissue. Treated tissues were placed, with the side opposing the direction of needle entry down, over the underside of a fully expanded young leaf removed from the first leaf pair of *Phaseolus vulgaris* 'White Half Runner' (a common host plant of *T. urticae*), and these leaves were, in turn, placed tissue side down over a shell vial which contained five female two spotted spider mites (Fig. 1). Shell vial closures were modified as mentioned previously. After 96 h the number of eggs laid per shell vial were counted. Two blocks, each including five shell vials of both treatments and a control, methylene chloride (without plant extract) applied to the perforated tissue, were run for each taxon. Data were transformed [sqrt (x)] and subjected to analysis of variance (version 6.10; SAS). Mean separation was accomplished using Fisher's protected LSD test.

Results and Discussion

Levels of resistance among *Buddleia* taxa

LEAF DISK BIOASSAY. The leaf disk bioassay indicated a range of susceptibility to the two-spotted spider mite among *Buddleia* taxa. The number of mites remaining on leaf disks and the number of eggs laid after 48 and 96 h provided similar resistance differences among taxa (Table 1). The number of eggs laid on leaf disks after 96 h was used to select taxa for further evaluation. More susceptible *Buddleia* taxa included many less pubescent taxa such as *B.*

Table 4. Predators per 6 cm² on *Buddleia* taxa from 8 May until 13 June field trials (error df = 2148, F = 3.91, P < 0.01 for averages).

Cultivar	Predators per 6 cm ² (no.)					
	8 May	14 May	25 May	3 June	13 June	Avg
Gloster ^y	0 a ^z	0.15 a	0.35 a	0.05 a	0 a	0.12 a
Sungold ^x	0 a	0 b	0.23 ab	0.13 a	0 a	0.09 a
Bonnie ^w	0.04 a	0 b	0.10 bc	0.05 a	0.10 a	0.07 ab
Pink Delight ^w	0 a	0.03 b	0.08 bc	0.05 a	0.05 a	0.04 ab
African Queen ^w	0 a	0 b	0.03 bc	0.13 a	0 a	0.04 ab
Cornwall Blue ^v	0 a	0 b	0 c	0.10 a	0.08 a	0.04 ab
Nanho White ^w	0 a	0.03 b	0.03 bc	0.03 a	0.03 a	0.03 b
Ile de France ^w	0 a	0 b	0.03 bc	0.03 a	0 a	0.03 b
Black Knight ^w	0 a	0 b	0.08 bc	0 a	0 a	0.02 b
Miss Ellen ^w	0 a	0 b	0 c	0.10 a	0 a	0.02 b
Alba ^u	0 a	0 b	0.03 bc	0 a	0 a	0.01 b

^zMeans in a column followed by the same letter are not significantly different ($\alpha = 0.05$ level) using Fisher's protected LSD test.

^y*Buddleia lindleyana*.

^x*Buddleia weyeriana*.

^w*Buddleia davidii*.

^v*Buddleia davidii* × *B. fallowiana*.

^u*Buddleia fallowiana*.

lindleyana and *B. japonica* while resistant taxa generally included more pubescent taxa such as *B. fallowiana* 'Alba' and *B. davidii* × *B. fallowiana* 'Cornwall Blue', thus revealing a possible cause for differences in spider mite susceptibility.

The exit of mites from leaf surfaces onto petroleum jelly was consistent with other research where mites departed leaf disks despite the presence of viscous compounds (Aina et al., 1972; Knipping et al., 1975; Mansour and Karchi, 1990; Wilde et al., 1991), but inconsistent with others where mites remained on leaf disks (East et al., 1992; Soans et al., 1973). The exit of mites from leaf disks is a nonpreference effect (Knipping et al., 1975) and may be considered to have little effect on a natural population of two-spotted spider mites if plants are separated (de Ponti, 1977a) or if plants are raised in a monoculture (de Ponti, 1977b) and so was not considered a major factor when considering *Buddleia* taxa for further evaluation.

SHELL VIAL BIOASSAY. Differences between and among resistant and susceptible taxa via the shell vial bioassay (Table 2) were essentially consistent with, though better defined than, results from the preliminary leaf disk bioassay. Eggs were laid primarily on leaf surfaces although some eggs were laid along the lip of shell vials. Differences in number of eggs laid per shell vial among taxa indicate gradients of resistance to the two-spotted spider mite among *Buddleia* taxa. The greatest number of eggs per shell vial were laid on the lower surface of *Buddleia davidii* 'African Queen' indicating a high degree of susceptibility to infestation by the two-spotted spider mite. The *Buddleia* taxa that showed the greatest resistance to two-spotted spider mite infestation, based on number of eggs laid per shell vial, were *B. fallowiana* Balif. 'Alba' and *B. davidii* × *B. fallowiana* 'Cornwall Blue' (Table 2) which had 7% and 14%, respectively, of the number of eggs laid on 'African Queen'. That 'Cornwall Blue', a cross between *B. davidii* and *B. fallowiana*, was one of the more resistant *Buddleia* indicates that *Buddleia* taxa could be hybridized to confer two-spotted spider mite resistance.

FIELD TRIAL. Field trials showed increased spider mite populations from early May until mid June (Table 3). After 13 June, spider mite populations declined abruptly and never rose above one two-spotted spider mite per block. Beneficial arthropods found within sampled areas included predatory mites, spiders, lady beetles, and lacewings. Beneficial arthropod numbers followed a trend similar to two-spotted spider mite numbers (Table 4). It is not known

whether predator numbers were highest on plants that were less resistant to spider mites due to larger quantities of spider mites, or to a lack of resistance factors which might affect predators on two-spotted spider mite resistant taxa. *Buddleia lindleyana* 'Gloster' maintained the highest average concentration of two-spotted spider mites from 8 May until 13 June. It is suspected that the reason for larger two-spotted spider mite populations on *B. lindleyana* 'Gloster' is that *B. lindleyana* is a semievergreen species and mites may overwinter in situ and initiate feeding and egg laying earlier in the season. Of the deciduous taxa, 'African Queen' and 'Sungold' maintained the highest concentrations of mites and eggs while *B. fallowiana* 'Alba' had the lowest concentration.

Two-spotted spider mites and eggs counted per 6 cm² leaf area per day on each taxon confirmed the results of the shell vial bioassay for deciduous *Buddleia* taxa. The ability of laboratory bioassays to predict field populations of spider mites is generally good (Rodriguez et al., 1983; Stoner and Stringfellow, 1967; Wilde et al., 1991), although each case in which a laboratory test is used to predict field populations must be examined separately due to inherent deviations in conditions between laboratory and field situations (de Ponti, 1977a). The relationship between the average number of mites per 6 cm² per day on deciduous taxa in field trials (8 May to 14 June) and average numbers of eggs laid

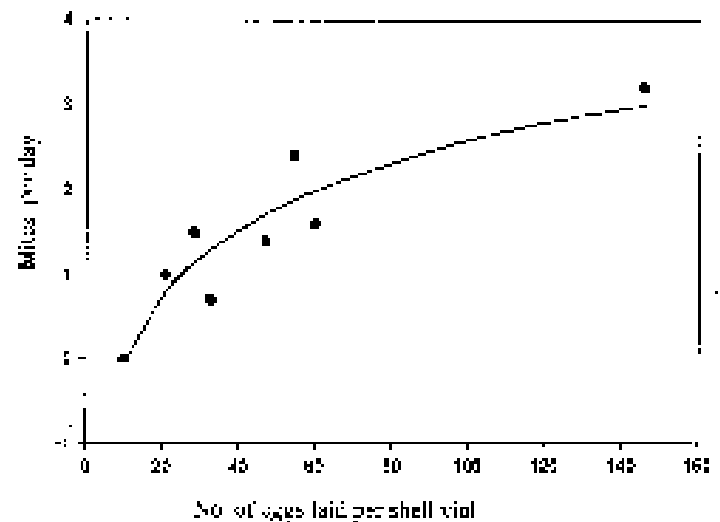


Fig. 2. Relationship between average number of two-spotted spider mites collected per day on an area 6 cm² in field plots and average number of two-spotted spider mite eggs laid per shell vial for eight deciduous *Buddleia* taxa. $y = -2.49 + 1.11(\ln x)$; $r^2 = 0.89$, $df = 6$, $F = 47.0$, $P < 0.01$.

Table 5. Trichome characteristics (trichome lengths, branches per square millimeter, and effective branch length) of nine *Buddleia* taxa (error df = 865; branch length F = 248.51, $P < 0.01$; branches per mm² F = 89.29, $P < 0.01$; EBL F = 99.55, $P < 0.01$).

Taxa	Branch length (mm)	Branches/mm ²	EBL (mm-mm ⁻²)
<i>B. fallowiana</i> Balif. Alba	0.399 a ²	419.9 c	168 a
<i>B. davidii</i> x <i>B. fallowiana</i> Cornwall Blue	0.357 b	398.3 cd	142 b
<i>B. davidii</i> Miss Ellen	0.280 c	395.3 d	111 c
<i>B. davidii</i> Ile de France	0.197 e	524.5 a	103 cd
<i>B. davidii</i> Black Knight	0.211 e	475.2 b	100 d
<i>B. weyeriana</i> Weyer Sungold	0.345 b	278.4 f	96 d
<i>B. davidii</i> Nanho White	0.260 d	321.4 e	84 e
<i>B. lindleyana</i> Gloster	0.074 g	772.8 a	57 f
<i>B. davidii</i> African Queen	0.170 f	229.2 g	39 g

²Means in a column followed by the same letter are not significantly

Correlation coefficients between egg laying data from the preliminary leaf disk bioassay and the average number of mites per 6 cm² per day counted in the field ($r^2 = 0.14$) were not as high as those between the shell vial bioassay and field data. The shell vial bioassay provided a more realistic test mechanism for assessing two-spotted spider mite resistance, as measured by fecundity, than the standard leaf disk bioassay because of a reduction in experimental error caused by viscous materials surrounding leaf disks and because of the orientation of leaves in a natural position for spider mite feeding. The shell vial bioassay system is easy to construct and provides legitimate results thus making it a viable alternative bioassay method for assessing two-spotted spider mite resistance in *Buddleia* taxa, and potentially in other taxa.

Resistance mechanisms

QUANTIFICATION OF PUBESCENCE. Pubescence was quantified by analyses of trichome branching and length and is expressed as mm trichome branches/mm² leaf surface area, or effective branch

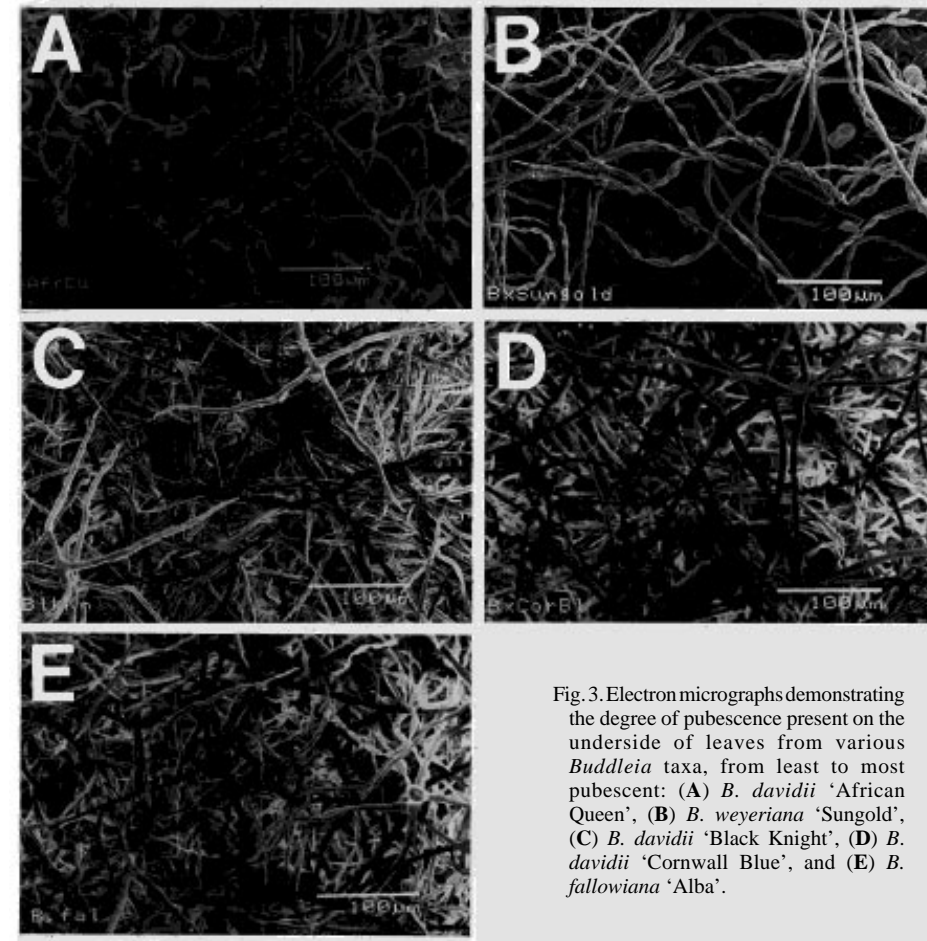


Fig. 3. Electron micrographs demonstrating the degree of pubescence present on the underside of leaves from various *Buddleia* taxa, from least to most pubescent: (A) *B. davidii* 'African Queen', (B) *B. weyeriana* 'Sungold', (C) *B. davidii* 'Black Knight', (D) *B. davidii* 'Cornwall Blue', and (E) *B. fallowiana* 'Alba'.

different ($\alpha = 0.05$) using Fisher's protected LSD test. after 96 h in the shell vial bioassay (Fig. 2) was observed. The high correlation ($r^2 = 0.89$) between data from the shell vial bioassay and the field trial supports the conclusion that the shell vial bioassay is a valid means of predicting the resistance of a *Buddleia* taxon to infestation by the two-spotted spider mite. *Buddleia lindleyana* 'Gloster' was not included in this correlation because of the confounding effect of a semi-evergreen species and should be expected to develop larger infestations of the two-spotted spider mite than deciduous taxa.

length (EBL). EBL measurements separated taxa by their relative quantities of pubescence (Table 5). Analyses of trichome branch lengths and densities using an electron microscope confirmed data collected using light microscopy as well as showing glandular trichomes not observed with light microscopy (Fig. 3). Plotting EBL versus number of eggs laid in the shell vial bioassay (Table 2) resulted in a 1/(square root EBL) correlation ($r^2 = 0.55$) demonstrating that more pubescent taxa (higher EBL) have fewer eggs laid on them than less pubescent taxa (Fig. 4). This result indicates that the quantity of pubescence present on *Buddleia* taxa can be used to predict two-spotted spider mite resistance. The fact that *B. davidii* x *B. fallowiana* 'Cornwall Blue' had the second greatest EBL, after *B. fallowiana* 'Alba', corroborates the hypothesis that resistance in *Buddleia*, correlated with EBL, is heritable.

REMOVAL OF PUBESCENCE. Removal of pubescence with facial peel resulted in an increase in oviposition for *Buddleia davidii* 'Black Knight' and *B. davidii* 'Cornwall Blue' (Table 6). This data supports the conclusion above, that increasing levels of pubescence result in lower mite fecundity. Removal of pubescence for assays against arthropod pests has, in the past, been accomplished using a razor blade (e.g., Hoffman and McEvoy, 1986) or other mechanical means to scrape the leaf surface. Removal of pubescence by scraping,

however, has the unfortunate side effect of damaging epidermal layers when trichomes are small. Advantages of using a A peel technique included minimal damage to leaf surfaces and almost complete pubescence removal. All pubescence was successfully removed from interveinal areas using facial peel, however, at the intersection of veins, there was frequently a small area of pubescence, ≈ 1 mm², that remained.

The decrease in two-spotted spider mite egg laying associated with increasing pubescence levels of *Buddleia* is consistent with

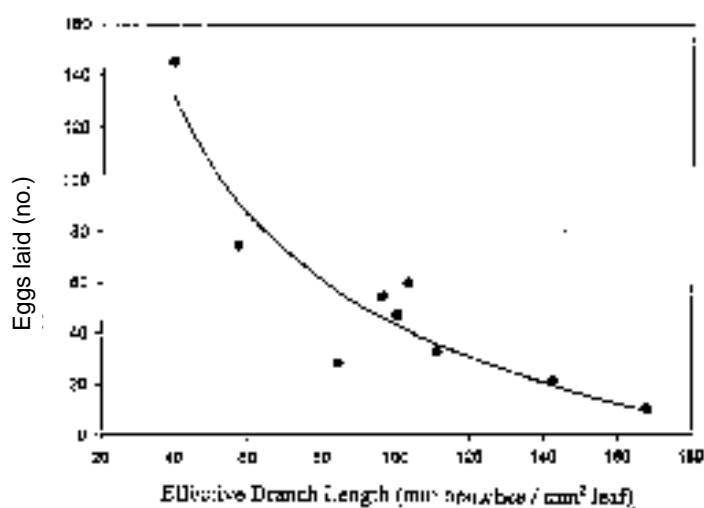


Fig. 4. Relationship between number of two-spotted spider mite eggs laid (five female mites, 96 h) in a shell vial bioassay and effective branch length (EBL) (mm trichome branches per square millimeter of leaf surface area) of nine *Buddleia* taxa. $y = -104.98 + 1478.83 (1/\sqrt{x})$; $r^2 = 0.55$, error $df = 36$, $F = 41.7$, $P < 0.01$.

findings in other crops including beach strawberry, *Fragaria chiloensis* Duch., where an increase in density of nonglandular trichomes decreased the number of eggs laid by the two-spotted spider mite (Luczynski et al., 1990) and cotton, *Gossypium hirsutum* L., where tall, highly branched trichomes conferred two-spotted spider mite resistance (Kamal and Elkassaby, 1965). In other crops, however, nonglandular trichomes have decreased resistance to the two-spotted spider mite. High densities of nonglandular trichomes increase two-spotted spider mite oviposition in hops, *Humulus lupulus* L., although mites do not necessarily show a preference for these more pubescent varieties (Peters and Berry, 1980). Likewise, *Fragaria xananassa* Duch. cultivars which are more pubescent tend to be more susceptible to attack by the two-spotted spider mite (Kishaba et al., 1972). Apple (*Malus x domestica* Borkh.) varieties with higher densities of trichomes are also more susceptible to infestations of *T. urticae* than less pubescent varieties (Yiem et al., 1993).

EFFECT OF LEAF SURFACE COMPOUNDS ON TWO-SPOTTED SPIDER MITE FECUNDITY. The modified shell vial bioassay forced mites to contact leaf surface extracts from experimental plants when mites fed or oviposited on the lower surface of the bean leaf. Methylene chloride extract from the more pubescent 'Cornwall Blue' resulted in an average of 43.0 eggs laid per shell vial. This was significantly less than the average of 98.4 eggs laid per shell vial when only methylene chloride was added to the tissue. Mites exposed to extract from 'African Queen' laid an average of 72.8 eggs per shell

Table 6. Effect of pubescence removal on the number of eggs laid per shell vial by five female two-spotted spider mites incubated with leaves from *Buddleia* cultivars (error $df = 17$; 'African Queen' $F = 1.65$, $P = 0.22$; 'Cornwall Blue' $F = 6.83$, $P < 0.01$; 'Black Knight' $F = 12.53$, $P < 0.01$; 'Miss Ellen' $F = 2.08$, $P = 0.16$).

Treatment	<i>B. davidii</i>			
	<i>B. davidii</i> African Queen	<i>x B. fallowiana</i> Cornwall Blue	<i>B. davidii</i> Black Knight	<i>B. davidii</i> Miss Ellen
Peeled	155.0 a ²	75.9 a	60.0 a	14.2 a
Nonpeeled	108.7 a	43.5 b	16.4 b	8.3 a

²Means in a column followed by the same letter are not significantly different ($\alpha = 0.05$) using Student's *t* test.

vial which was statistically similar to 'Cornwall Blue' and the control. *Buddleia* species have glandular trichomes (Rogers, 1986) whose constitutory compounds are unknown but which may affect the two-spotted spider mite in a deleterious way.

The identification of two-spotted spider mite resistant *Buddleia* taxa indicates that progeny could be bred for greater resistance. Resistance to the two-spotted spider mite in *Buddleia* taxa is correlated with a particular taxon's quantity of nonglandular pubescence, thus making quantity of pubescence an effective indicator of practical resistance to the two-spotted spider mite. There is also evidence to indicate that chemical constituents are present on the surface of leaves, and potentially in trichomes, which affect the ability of two-spotted spider mites to infest a given *Buddleia* taxon. The commercial production of *Buddleia* taxa that are more resistant to the two-spotted spider mite would reduce aesthetic damage and pesticide inputs required to grow this crop.

Literature Cited

- Aina, O.J., J.G. Rodriguez, and D.E. Knavel. 1972. Characterizing resistance to *Tetranychus urticae* in tomato. *J. Econ. Entomol.* 65:641-643.
- Baker, J. R. 1980. Insects and related pests of shrubs. N.C. Coop. Ext. Serv. Publ. AG-189.
- Chin, S.F., S. Lin, and C.Y. Hu. 1947. Toxicity study of insecticidal plants in southwest China. *Chem. Abstr.* 41:2201-2202.
- de Ponti, O.M.B. 1977a. Resistance in *Cucumis sativus* L. to *Tetranychus urticae* Koch. 2. Designing a reliable laboratory test for resistance based on aspects of the host-parasite relationship. *Euphytica* 26:641-654.
- de Ponti, O.M.B. 1977b. Resistance in *Cucumis sativus* L. to *Tetranychus urticae* Koch. 1. The role of plant breeding in integrated control. *Euphytica* 26:633-640.
- Dirr, M.A. 1998. Manual of woody landscape plants. Stipes Publishing Co., Champaign, Ill.
- East, D.A., J.V. Edelson, E.L. Cox, and M.K. Harris. 1992. Evaluation of screening methods and search for resistance in muskmelon, *Cucumis melo* L., to the two-spotted spider mite, *Tetranychus urticae* Koch. *Crop Prot.* 11:39-44.
- Ferrer, R.M.G., J.C. Scheerens, and W.A. Erb. 1993. In vitro screening of 76 strawberry cultivars for two-spotted spider mite resistance. *HortScience* 28:841-844.
- Herron, G., L. Jiang, and R. Spooner-Hart. 1996. A laboratory-based method to measure relative pesticide and spray oil efficacy against broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae). *Expt. Appl. Acarol.* 20:495-502.
- Hoffman, G.D. and P.B. McEvoy. 1986. Mechanical limitations on feeding by meadow spittlebug, *Philaenus spumarius* (Homoptera: Cercopidae), on wild and cultivated host plants. *Ecol. Entomol.* 11:415-426.
- Hudson, W.G., S.K. Braman, R.D. Oetting, and B.L. Sparks. 1996. Ornamental, lawn, and turf insects, p. 20-22. In: R.M. McPherson, G.K. Douce, and D.G. Riley (eds.). Summary of losses from insect damage and costs of control in Georgia 1995. *Ga. Agr. Expt. Sta. Spec. Publ.* 90.
- Kabir, K.H., R.B. Chapman, and D.R. Penman. 1996. Mite bioassays with spider mites (Acari: Tetranychidae): Effect of test design and sample size on the precision of lethal concentration estimates. *Expt. Appl. Acarol.* 20:483-494.
- Kamal, S.A. and F.Y. Elkassaby. 1965. Relative resistance of cotton varieties to spider mites, leafhoppers, and aphids. *J. Econ. Entomol.* 58:209-212.
- Kishaba, A.N., V. Voth, A.F. Howland, R.S. Bringham, and H.H. Toba. 1972. Two-spotted spider mite resistance in California strawberries. *J. Econ. Entomol.* 65:117-119.
- Knipping, P.A., C.G. Patterson, D.E. Knavel, and J.G. Rodriguez. 1975. Resistance of cucurbits to two-spotted spider mite. *Environ. Entomol.* 4:507-508.
- Luczynski, A., M.B. Isman, D.A. Raworth, and C.K. Chan. 1990. Chemical and morphological factors of resistance against the two-spotted spider mite in beach strawberry. *J. Econ. Entomol.* 83:564-569.

- Mansour, F. and Z. Karchi. 1990. The evaluation of antibiosis of selected lines for resistance of melon to the carmine spider mite *Tetranychus cinnabariensis* (Acari: Tetranychidae). *Bul. Entomol. Res.* 80:345–347.
- Maunder, M. 1987. Notes on tender species of *Buddleia*. *The Plantsman* 9:64–80.
- Metcalf R.L. and R.A. Metcalf. 1993. *Destructive and useful insects*, 5th ed. McGraw Hill, New York.
- Owen, D.F. and W.R. Whiteway. 1980. *Buddleia davidii* in Britain: History and development of an associated fauna. *Biol. Conserv.* 17:149–155.
- Peters, K.M. and R.E. Berry. 1980. Effect of hop leaf morphology on two-spotted spider mite. *J. Econ. Entomol.* 73:235–238.
- Roder, E., H. Wiedenfeld, and A. Hoenig. 1985. Isolation of a new tricyclic piperidine alkaloid from *Buddleia davidii*. *Planta Med.* 51:164–165.
- Rodriguez, J.G., D.A. Reicosky, and C.G. Patterson. 1983. Soybean and mite interactions: Effects of cultivar and plant growth stage. *J. Kan. Entomol. Soc.* 56:320–326.
- Rogers, G.K. 1986. The genera of Loganiaceae in the southeastern United States. *J. Arnold Arbor.* 67:143–185.
- Schuster, M.F., F.G. Maxwell, J.N. Jenkins, and W.L. Parrott. 1972. Mass screening seedlings of *Gossypium* sp. for resistance to the two-spotted spider mite. *J. Econ. Entomol.* 65:1104–1107.
- Shoeb, H.A., A.M. Mousa, M.M. El Sayed, and A.M. Emam. 1991. Laboratory evaluation of some plants as molluscicidal agent. *Egypt. J. Bilharziasis.* 13:189–194.
- Soans, A.B., D. Pimentel, and J.S. Soans. 1973. Resistance in cucumber to the two-spotted spider mite. *J. Econ. Entomol.* 66:380–382.
- Stermitz, F.R. and G.R. Harris. 1985. Reappraisal of the structure for the alkaloid buddamin from *Buddleia davidii*. *Tetrahedron Lett.* 26:5251–5252
- Stoner, A.K. and T. Stringfellow. 1967. Resistance of tomato varieties to spider mites. *Proc. Amer. Soc. Hort. Sci.* 90:324–329.
- Wilde, G., W. Thomas, and H. Hall. 1991. Plant resistance to two-spotted spider mite (Acari: Tetranychidae) in raspberry cultivars. *J. Econ. Entomol.* 84:251–255.
- Yiem, M.S., J.H. An, and Y.I. Lee. 1993. Relationships between morphological characteristics of apple leaf and resistance to two-spotted spider mite (*Tetranychus urticae* Koch). *RDA J. Agr. Sci. Hort.* 35:464–470.
- Yoshida, T., J. Nobuhara, N. Fujii, and T. Okuda. 1978. Studies on the constituents of *Buddleia* species. II. Buddledin C, D and E, new sesquiterpenes from *Buddleia davidii* Franch. *Chem. Pharm. Bul.* 26:2543–2549.
- Zhang, X., Y. Xi, W. Zhou, and M. Kay. 1993. *Cleopus japonicus*, a potential biocontrol agent for *Buddleia davidii* in New Zealand. *N.Z. J. For. Sci.* 23:78–83.