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Robert W. Cruden
University of Iowa, robert-cruden@uiowa.edu

Kristina K. Baker
University of Iowa

Thomas E. Cullinan
University of Iowa

Karen A. Disbrow
University of Iowa

Kelly L. Douglas
University of Iowa

See next page for additional authors

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The Mating Systems and Pollination Biology of Three Species of *Verbena* (Verbenaceae)

ROBERT W. CRUDEN[1], KRISTINA K. BAKER, THOMAS E. CULLINAN, KAREN A. DISBROW, KELLY L. DOUGLAS, JOHN D. ERB, KENNETH J. KIRSTEN, MARY L. MALIK, ELIZABETH A. TURNER, JONATHON A. WEIER, and SHERRY R. WILMOT

Iowa Lakeside Laboratory, Milford, IA 51351 and Program in Ecology and Evolutionary Biology and Department of Botany, University of Iowa, Iowa City, IA 52242

Because their flowers can be cross- and/or self-pollinated *Verbena stricta, V. hastata* and *V. urticifolia* are facultatively xenogamous. We suggest the flowers can be cross-pollinated because 1) the fruit set of caged plants was substantially lower than that of open-pollinated plants, i.e. pollinators were necessary for typical fruit set and 2) the flowers of each species attracted a diverse array of hymenopteran, dipteran and lepidopteran pollinators that were capable of moving pollen between plants. Self-pollination was low due to the spatial separation of anthers and stigmas and/or an angled corolla that decreased the likelihood of pollen dropping from the anthers onto the stigma. However, the limited ability of flowers to self-pollinate was supplemented by the intrafloral movement of pollen by thrips. In addition, both the pollen-ovule ratios and pollination efficiencies of these species were consistent with those of other facultatively xenogamous species, and the available data were consistent with each species being self-compatible. Finally, we discuss a protocol for distinguishing between self-pollination and the intrafloral movement of pollen by thrips.
INDEX DESCRIPTORS: facultative xenogamy, plant mating systems, thrips pollination, *Verbena*.

In northwest Iowa four species of *Verbena* are relatively common and constitute a series with respect to corolla size. We hypothesized that the differences in corolla size should be associated with differences in mating systems and pollinators. Species with larger corollas and showy inflorescences, i.e., *V. stricta* Vent. and *V. hastasta* L. should be facultatively xenogamous (cross- and/or self-pollinating) or xenogamous (cross-pollinating) and those with small corollas and inconspicuous inflorescences, i.e., *V. urticifolia* L. and *V. bracteata* Lag. & Rodr., should be facultatively autogamous (primarily self-pollinating). Previous work suggests that *V. stricta* is facultatively xenogamous and that the latter two species are either facultatively xenogamous or facultatively autogamous (Cruden 1977; Perkins et al. 1975). Perkins et al. (1975) reported substantial differences in the seed set of open-pollinated and caged flowers of *V. stricta* (87.6 vs. 7.5%) and *V. bracteata* (66.5 vs. 21.9%) and smaller differences in *V. halei* Small (68.0 vs. 54.5%) and *V. urticifolia* (66.5 vs. 47.3%). The low seed sets they reported for open-pollinated and caged plants are not typical of facultatively xenogamous or facultatively autogamous species, whose fruit sets are usually in excess of 85% (Cruden and Westley 1991). The pollen-ovule ratio of *V. bracteata* is characteristic of a facultatively autogamous species (Cruden 1977).

Our objective was to describe and compare the mating systems and pollinators of *V. stricta, V. hastata* and *V. urticifolia*. We used an experimental protocol that was designed to distinguish between three mating systems, xenogamy, facultative xenogamy, and facultative autogamy (Cruden and Lyon 1989). We included in our protocol a treatment designed to distinguish between autogamy and thrips mediated movement of pollen within flowers because thrips pollination would provide an over estimate of the ability of flowers to self-pollinate. We examined floral morphology to see if it played a role in the ability of the flowers to self-pollinate. We also determined pollen-ovule ratios and pollination efficiencies because they are correlated with mating systems (Cruden 1977, 1991, Cruden and Lyon 1989). Flower visitors were collected and their proboscises examined for the presence of pollen.

STUDY SITES

Observations were made at sites in Dickinson County, Iowa and Houghton County, Michigan. Pollinators were observed and collected on *V. stricta* at Iowa Lakeside Laboratory, Route 86, 5.5 km S Route 9 (Sec. 23, Lakeville Twp.) and a small, dry hill to the north of Route 9, 1.1 km east of Route 86 (Sec. 36, Diamond Lake Twp.) and on *V. hastata* south of the hardtop road by Jemmerson Slough, 0.5 km E of Route 9 (Sec. 6, Center Grove Twp.), a marsh along Route 9, ca 4 km W of Route 86 (NW corner Sec 5, Lakeville Twp.), and Three-Corner Pond, gravel road ca 1 km W of Route 86 (NE corner of Sec. 3, Okoboji Twp.). In addition, pollinators were observed and collected in a small population along Kidney Creek, Houghton County, Michigan, ca 0.5 km NW of the road to Perch Lake (NW corner Sec 21, Bates Twp.). The only population of *V. urticifolia* studied was at Iowa Lakeside Laboratory. The experiments designed to test the ability of flowers to self-pollinate were conducted at Iowa Lakeside Laboratory (*V. stricta* and *V. urticifolia*) and Three-Corner Pond (*V. hastata*).

MATERIALS AND METHODS

We used two treatments to distinguish between the ability of flowers to self-pollinate and/or thrips to move pollen within flowers. The inflorescences of one set of plants of both *V. hastata* and *V. stricta* were enclosed in bags of mosquito netting and the inflorescences of a second set of plants were sprayed with malathion, tanglefoot was applied to the base of the inflorescence to keep insects from crawling up into the inflorescence and the plants were covered with cages made of mosquito netting. These inflorescences were sprayed with malathion every other day during the experiment. Fruit set was determined from material collected two weeks later.

A second set of plants was used to determine pollination success. Plants of *V. stricta* and *V. urticifolia* were caged and inflorescences of *V. hastata* were bagged. One group of plants of each species received a single application of malathion at the start of the experiment, which was insufficient to eradicate all the thrips (thrips were found in the flowers of *V. hastata*). After 12 days the inflorescences were collected, placed in 95% ethanol, and subsequently the stigmas were scored for pollen grain number (see below). The data were arcsine-square root transformed and examined with a one-way analysis of variance (=ANOVA) (Ryan, Joiner, and Ryan 1985) and the means compared with Student-Newman-Keuls test (Sokal and Rohlf 1969). The latter test compares the least significant range (=LSR) with an observed range and if the latter is greater the comparison is statisti-

[1]Co-authors in alphabetical order; write RWC, Dept. of Botany for reprints.

Contribution No. 443 from Iowa Lakeside Laboratory

cally significant at the indicated level.

Pollen-ovule ratios (P/O's) were determined by carefully removing one or two anthers from an unopened flower in a droplet of cotton-blue in lacto-phenol, separating the pollen grains from the anther(s), removing the fragments of anther wall from the stain, covering with a small part of a 22 mm coverslip, and counting the number of pollen grains at 40X. The preparation and counting were done on a grided slide (Lovins Micro-slide Field Finder). Because there are 4 anthers and 4 ovules the estimate of pollen per anther is also the P/O. A single flower from each of 10 plants was used.

One flower from each of 12-14 plants was used to determine the width and height of the corolla and length of the corolla tube. The distance to the base of the lowest anther was measured from the base of the corolla tube. The distance between the stigma and the lowest anther was estimated by subtracting the length of the pistil from the distance to the base of the lowest anther.

Nectar Measurements:

Nectar for measuring volumes and sugar concentrations was collected in one microliter pipettes and the volume determined by dividing the length of the nectar column by the length of the capillary tube. The sugar concentration was measured with a Bellingham and Stanley Pocket Refractometer. To get sufficient nectar to determine sugar concentration nectar from several flowers was combined.

Flower Longevity:

To determine the life span of a flower we placed a thread above and below those flowers that opened on a single day and censused them periodically until they dropped from the plant. Twenty plants each of *V. stricta* and *V. hastata* were used. For *V. stricta* two sets of flowers were followed for 5 days, one set for 3 days and one set for 2 days. Each of three sets of *V. hastata* flowers were followed for three days. Two to three flowers on each of 15 plants of *V. urticifolia* were marked on each of four mornings and examined that afternoon and the following morning.

Pollination efficiency:

Pollination efficiency was determined from an estimate of the number of pollen grains per stigma divided by an estimate of the number of pollen grains per flower, i.e., 4 times the P/O. We used 0, 7, and 16 as the estimates of the mean number of pollen grains per stigma in the 0, 1-12, and 13-20 classes (Table 3), respectively, which are the median numbers for those classes. We assumed that the distribution of pollen grains was more or less even across each of these classes. The estimate for the number of pollen grains per stigma for the >20 class was obtained by counting those grains that were easily observed on three stigmas from each of 10 plants and calculating the mean for each plant. This provided a minimal estimate of pollen number because some pollen grains in a dense mass were hidden and not counted. The estimates for each class (% stigmas × #pollen grains/stigma) were summed and divided by the number of pollen grains per flower. The numbers of pollen grains were determined from stigmas prepared for fluorescence microscopy. The pistils were preserved in 95% ethanol and subsequently placed in 1N NaOH for 8-16 hours and stained overnight in 0.1% aniline blue in 0.1 K_3PO_4 then examined with a fluorescence microscope (Kho and Baer 1968).

Means ± the standard error of the mean are reported for all measurements.

RESULTS

The flowers of all the species were borne on many flowered indeterminate spikes. Large plants bore numbers of spikes. The flowers of *V. stricta* and *V. hastata* were blue and formed quite showy clusters. The corollas were salverform and the corolla tubes were angled above the level of the stigma and the expanded portion of the flower, i.e., the limb, faced outward. In each species the stigma was usually below the base of the lowest anther (Table 1). Occasionally the pistil was sufficiently long that the stigma contacted the lowest anther. This occurred more frequently in *V. stricta* than in the other species. The small, white flowers of *V. urticifolia* were notable because they produced a sweet odor. The inflorescences of this species were relatively inconspicuous because each branch bore only two to four flowers at a time.

Mating System:

The fruit set of open-pollinated flowers was over 90% in each species and the exclusion of pollinators reduced fruit set 55% in *V. stricta* and 81% in *V. hastata* (Table 2) and the malathion treatment reduced fruit set further. An ANOVA showed that in *V. stricta* the differences in the percentage of flowers setting 4 seeds were statistically significant ($F = 50.20$, $df = 2, 40$, $p < .001$) as was the number of flowers not producing a fruit ($f = 44.44$, $df = 2, 40$, $p < .001$). In both comparisons the difference between open-pollinated flowers and the two treatments was significant ($p << .01$). The percentage of bagged flowers setting 4 seeds was not statistically different from the percentage of flowers on sprayed + caged plants setting 4 seeds ($LSR_{\alpha=.05} = 16.16 > 12.27$). In contrast, the application of malathion increased significantly the number of flowers setting no seed relative to bagged flowers ($LSR_{\alpha=.05} = 16.27 < 17.48$).

The pattern for *V. hastata* was the same (Table 2). The ANOVA's were highly significant for flowers setting 4 seeds ($F = 169.25$, $df = 2, 38$, $p << .001$) and those setting 0 seeds ($F = 167.71$, $df = 2, 38$, $p << .001$). Excluding pollinators resulted in a large and significant decrease in the number of flowers that set 4 seeds and significantly increased the number of flowers setting no seed. The difference between bagged and sprayed + caged flowers setting 4 seeds was not significant ($LSR_{\alpha=.05} = 12.24 > 9.31$) and again the application of malathion and caging reduced fruit set significantly relative to the bagged flowers ($LSR_{\alpha=.01} = 16.24 < 17.04$).

In late August, toward the end of the flowering season, a parallel experiment was run to examine pollination success (Table 3). The exclusion of pollinators significantly decreased the numbers of pollen grains deposited on the stigmas of both *V. stricta* ($F = 74.19$, $df = 2, 26$, $p < .001$) and *V. hastata* $F = 227.37$, $df = 2, 27$, $p << .001$) and increased significantly the percentage of stigmas that received no pollen ($F = 65.48$, $df = 2, 26$, $p << .001$ and $F = 249.56$, $df = 2, 27$, $p << .001$; *V. stricta* and *V. hastata*, respectively). In both species the difference between caged/bagged and spray + caged/bagged treatments was not statistically significant. This probably reflects the presence of thrips in the flowers of the sprayed plants.

In *V. urticifolia* the spray + cage treatment decreased significantly the percentage of flowers receiving more than 20 pollen grains and increased significantly the percentage receiving no pollen grains. We scored flowers from 9 caged plants ($n = 23.9 \pm 1.9$ flowers/plant) for the presence of pollen grains on the stigma; only 36.2±9.3% of the stigmas had received pollen grains.

The ability of flowers to self-pollinate during flower opening was tested by carefully removing the corolla from the rest of the flower

Table 1. Lengths ($\bar{X} \pm$S.E. in mm) of flora parts and distance between the stigma and lowest anther.

	V. stricta (n = 14)	*V. hastata* (n = 14)	*V. urticifolia* (n = 12)
Corolla length	9.8±0.4	4.4±0.1	2.6±0.1
Corolla width	8.5±0.3	4.5±0.2	2.6±0.1
Corolla tube length	5.5±0.2	4.1±0.1	2.0±0.0
Height of stigma	2.9±0.1	2.4±0.0	1.3±0.0
Distance to lowest anther	3.2±0.1	2.9±0.1	1.4±0.0
Distance between stigma and lowest anther	0.25±0.06	0.45±0.06	0.08±0.01

Table 2. Percentage of flowers (±S.E.) setting seed in open-pollinated, bagged, and sprayed + caged plants of *V. stricta*, *V. hastata*, and *V. urticifolia*. One inflorescence from each plant was collected. For *V. stricta* and *V. hastata* 20 flowers from each open-pollinated inflorescence were scored and 15 flowers from each bagged and sprayed + caged inflorescence were scored. The open-pollinated data from *V. urticifolia* was based on 9.1±0.5 flowers per plant.

		Number of seeds set per flower				
	n=	0	1	2	3	4
V. stricta						
Open-pollinated	20	6.5±3.7	0.8±0.8	2.0±1.0	0.8±0.8	90.5±4.0
Bagged	15	61.8±7.1	4.4±1.4	3.6±1.3	1.3±0.7	28.9±7.2
Sprayed + Caged	8	84.2±6.5	0.8±0.8	1.8±1.2	1.8±1.2	11.8±5.9
V. hastata						
Open-pollinated	20	5.0±1.8	0.0	0.5±0.3	1.5±0.6	93.0±2.2
Bagged	15	86.2±4.5	5.8±1.7	0.0	0.0	8.0±4.2
Sprayed + Caged	6	100.0±0.0	0.0	0.0	0.0	0.0
V. urticifolia						
Open-pollinated	16	0.0	1.4±1.0	6.7±2.4	17.3±3.9	74.4±5.7

and checking the stigmatic surface for pollen grains. The stigmas of 7/40 *V. stricta* flowers were pollinated. One flower from each of 29 plants of *V. hastata* at Jemmerson Slough and one flower from each of 54 plants at Three-Corner Pond were examined. At each site a single stigma had received pollen grains, 1 and 3 respectively. We examined 67 flowers from 23 plants (2.9±0.2/plant) of *V. urticifolia* and 12 stigmas were pollinated. Six had received 1 pollen grain, 4 had received 3, and the other two, 2 and 4 grains, respectively. Most of these pollen grains had germinated and in half the pollen tubes had already reached the ovary.

Although no direct test of compatibility was done the ability of caged plants of all species to set fruits indicated that they were, to some degree, self-compatible. The observation of pollen tubes in just opened flowers of *V. urticifolia* also was consistent with self-compatibility.

The pollen-ovule ratos of *V. stricta*, *V. hastata*, and *V. urticifolia* were 579.0±43.2, 403.0±15.8, and 291.3±11.2, respectively. One or two individuals of each species produced >10% sterile pollen grains.

Flower Longevity:

Most of the flowers of each species opened between 0930 and 1100 h. All of the flowers of *V. stricta* (n=532) lasted 24 hours and 74% (312/419) lasted through the second day, i.e., 36 hours. At the start of the third day (48 hours) 37% (141/379) of the flowers remained, 9% (25/265) were present at the start of day 4 and most of those fell during the day. No flowers lasted more than four days. The differences in sample sizes reflects the length of time the several groups of flowers were followed as well as the frequency of censusing (see methods). The flowers of *V. hastata* are shorter lived; 85% (n=385) were present at the start of day two and 23% were left at the end of the day. Only 4% remained at the start of day 3 and all had fallen by the end of the day. The flowers of *V. urticifolia* (n=146) were open less than one day as no flower lasted 24 hours. The flowers of all the species were observed on warm, sunny days so that we measured the longevity of flowers that were likely to have been visited. Flowers may last longer in conditions that limit the activity of flower visitors.

Nectar Production:

The flowers of all three species produced nectar. Newly opened flowers of both *V. stricta* (.09 ul) and *V. hastata* (.02 ul) contained less nectar than older flowers (.445 and 0.31 ul, respectively). The sugar concentration of the nectar from *V. stricta* was 37.5±1.2% (n=13). The flowers of *V. urticifolia* produce quite small amounts of nectar and we were unable to obtain accurate measurements of the volume.

To test the idea that nectar production would cease following successful pollination, second day flowers of *V. stricta* were scored for amount of nectar and presence of pollen grains on the stigmas. We predicted that flowers with large amounts of nectar (>.12 ul) would

Table 3. Percentage of stigmas (±S.E.) with different numbers of pollen grains. The flowers were open-pollinated, caged, and sprayed with malathion then caged. For *Verbena stricta* a mean of 46.3±1.0 flowers were sampled from each open-pollinated plant (n=11), 75.0±13.2 flowers from each caged plant (n=9), and 140.2±28.2 flowers from each sprayed+caged plant (n=9). For *v. hastata* we examined 52.3±2.3 flowers from each open-pollinated plant (n=10), 121.8±8.8 flowers from each bagged plant (n=10), and 203.6±13.4 flowers from each sprayed+bagged plant (n=10). For *V. urticifolia* 3.6±0.5 flowers were sampled from 14 open-pollinated plants and 24.0±4.0 flowers were sampled from 12 sprayed+caged plants.

	Number of Pollen Grains per Stigma			
	0	1-12	13-20	>20
V. stricta				
Open-pollinated	2.3±1.0	11.5±3.6	4.0±1.2	81.8±4.9
Caged	49.3±6.1	34.4±4.1	6.7±2.1	9.7±2.5
Sprayed+Caged	59.9±6.2	28.2±4.1	3.6±1.1	7.3±3.0
V. hastata				
Open-pollinated	0.5±0.4	4.0±0.8	4.9±1.5	90.6±2.1
Bagged	57.9±4.9	15.7±2.2	6.0±1.6	20.5±3.7
Sprayed+Bagged	54.5±1.7	20.2±1.5	4.5±0.7	21.0±1.9
V. urticifolia				
Open-pollinated	0.0	0.0	0.0	100.0
Sprayed+Caged	41.7±6.1	43.1±5.1	5.1±1.9	8.4±2.8

Table 4. Flower visitors and/or pollinators in populations of *Verbena hastata* in northwestern Iowa and the Upper Peninsula of Michigan. JS = Jemmerson Slough, TCP = Three-Corner Pond, R9M = Marsh, Rt 9, W Rt 86, KC = Kidney Creek; f = female, m = male, w = worker; P = pollen on proboscis or galea, NP = no pollen;

HYMENOPTERA:
- ANDRENIDAE: *Calliopsis nebraskensis* Crawford (JS: 3f-NP; TCP: 4f-P, 1f-NP, 1m-P; R9P:1f-NP, 1m-P).
- ANTHOPHORIDAE: *Melissodes trinodis* Robertson (TCP: 1f-P); *Melissodes* cf *subillata* LaBerge (JS: 1f-P); *Melissodes* sp. (TCP: 1m-P).
- APIDAE: *Apis mellifera* L. (JS: 3w-P; TCP: 6w-P; R9M); *Bombus vagans* Smith (JS: 1w-P; R9M: 1w-P); *Bombus pennsylanicus* (Degeer) (KC: 1w-P); *Bombus affinis* Cresson (KC: 1w-P); *Psithyrus ashtoni* (Cresson) (KC: 1w-P).
- HALICTIDAE: *Augochlorella striata* Provancher (JS: 1f-P); *Agapostemon virescens* (Fabricius) (TCP: 1f).
- MEGACHILIDAE: *Hoplitis* sp. (JS: 1f-P); *Megachile* sp. (TCP: 1f-P); Megachile sp. (TCP: 1f-P).
- XYLOCOPIDAE: *Ceratina calcarata* Robertson (JS: 1f-P, 1f-NP).

DIPTERA
- BOMBYLIIDAE: *Exoprosopa fasciata* Macquart (TCP: 1P).
- SYRPHIDAE: *Eristalis tenax* L. (JS: 3P; R9M: obs); *Eristalis latifrons* (Loew) (JS: 1P, 1NP); *Tropidia quadrata* (Say) (JS: 1P, 2NP; TCP: 1P, 1NP).
- TACHINIDAE: *Archytas apicifer* (Walker) (TCP: 2P; R9M: 3P).

LEPIDOPTERA
- DANAIDAE: *Danaus plexippus* L. (JS; R9M; TCP).
- NYMPHALIDAE: *Vanessa atalanta* L. (JS).
- LYCENIDAE: *Everes comyntas* Godart (TCP: 1-NP).
- PIERIDAE: *Pieris rapae* L. (JS: 1-P); *Colias eurythene* Boisduval (TCP: 2-P; R9M); *Colias philodice* Latreille (TCP: 1-NP).
- CTENUCHIDAE: *Ctenucha* cf *virginica* (Esp.) (TCP: 3-P).
- NOCTUIDAE: *Anagrapha falcifera* (Kby.) (= *Autographa simplex* (Gn.) (TCP: 1-P); *Feltia subgothica* (Haw.) (TCP: 2-P).

be unpollinated and those with small amounts of nectar (<.12 ul) would be pollinated. Ten second day flowers in each nectar class were scored for pollination and each had pollen tubes in the ovary, i.e., fertilization had probably occurred.

Flower visitors:

During the course of the flowering season the flowers of *V. hastata* were visited by a diverse array of bees, flies, butterflies and moths (Table 4). The proboscises of these species were long enough to reach the bottom of the corolla tube and some individuals of each species had pollen on their proboscis. From mid to late July, most of the visitors were bees and flies and just a few butterflies were observed. *Apis mellifera* was always the most common visitor. On July 20-22 we censused the bees visiting flowers at Jemmerson Slough seven times. During the two censuses in which we counted all of the bees, *Apis* constituted 70-80% of the bees (32/45 and 34/41) visiting the flowers. Of the other bees observed during the seven censuses 44 of 78 were *Calliopsis*, which may collect pollen primarily from species of *Verbena* (Mitchell 1959; Krombein et al. 1979). We did not include the various species of flies in the censuses but the two species of *Eristalis* were about as common as *Calliopsis*. At the marsh west of Route 86, *Apis*, *Calliopsis* and *Archytas apicifer* (Tachinidae) were common flower visitors and important pollinators. A census at this site provided the following data: 81 *Apis*, 3 *Bombus*, 8 *Calliopsis*, 16 *Archytas*, 1 *Eristalis tenax*, 1 monarch butterfly and 1 Orange Sulphur. In late August (26-27) large numbers of pierid butterflies and moths visited the flowers of the Three-Corner Pond population and few bees were observed.

Table 5. Pollinators and putative pollinators of *Verbena stricta* in northwestern Iowa. ILL = Iowa Lakeside Laboratory, R9H = hill, Rt. 9, 1.5 km E Rt. 86; TCP = Three-Corner Pond; P = pollen on proboscis, NP = no pollen; f = female, m = male, w = worker.

HYMENOPTERA:
- ANDRENIDAE: *Calliopsis andreniformis* Smith (R9H: 1f-P); *Calliopsis nebraskensis* Crawford (ILL: 1f-NP).
- ANTHOPHORIDAE: *Melissodes* sp. (R9H:1f-P).
- APIDAE: *Apis mellifera* L. (ILL: 2w-P, 3-NP; R9H; TCP); *Bombus nevadensis* Cresson (ILL: 1f-P, 1w-NP); *Bombus vagans* Smith (ILL: 2w-P, 1w-NP); *Bombus bimaculatus* Cresson (R9H).
- HALICTIDAE: *Lasioglossum* cf *leucozonium* (Schrank) (R9H: 3m-P).
- MEGACHILIDAE: *Hoplitis* sp. (R9H:1f-NP).
- XYLOCOPIDAE: *Ceratina calcarata* Robertson (ILL: 1f-NP).
- POLISTIDAE: *Polistes* sp. (ILL: 1f-NP).

DIPTERA:
- SYRPHIDAE: *Tropidia quadrata* (Say) (ILL: 1-P; R9H: 1-NP).

LEPIDOPTERA
- DANAIDAE: *Danaus plexippus* L. (ILL).
- NYMPHALIDAE: *Vanessa atalanta* L. (R9H).
- PIERIDAE: *Pieris rapae* L. (ILL, R9H); *Colias* sp. (ILL).

The flowers of *V. stricta* were visited primarily by larger bees and butterflies (Table 5). The few solitary bees that we observed included two species of *Calliopsis*. Pollen was found on the proboscis of most of the individuals that we collected. The flowers of *V. urticifolia* were visited by a number of small, solitary bees that are known to be polylectic (Table 6).

Pollination Success and Efficiency:

In late July flowers of *V. stricta* were collected prior to the initiation of pollinator activity on day 2 and the stigmas were scored for the presence of pollen grains. The stigmas of all the flowers (n = 38) had received pollen grains. In late August flowers were collected from 20 individuals and 97% of the flowers had been pollinated (Table 2). Pollination success was equivalent in *V. hastata* (99.5%) and *V. urticifolia* (100%) (Table 3).

The estimates of pollination efficiency for the three species were consistent with those of other facultatively xenogamous species, i.e., *V. stricta* - 3.8%, *V. hastata* - 3.0%, and *V. urticifolia* - 5.0%. Estimates of stigmatic pollen loads for the stigmas receiving more than 20 pollen grains for the three species were 107.1, 60.4, and 57.5, respectively.

The number of pollen grains remaining in the anthers of 38 flowers of *V. stricta* were counted. The range was 1-182 and the mean was 44.4±8.9 pollen grains per flower. On average approximately 98% of the pollen was removed by flower visitors.

Table 6. Visitors to the flowers of *V. urticifolia* at Iowa Lakeside Laboratory. f = female, m = male; P = pollen present, NP = no pollen present.

HYMENOPTERA
- ANTHOPHORIDAE: *Epeolus bifasciatus* Cresson (1f-P).
- HALICTIDAE: *Dialictus* #1 (1m-P); *Dialictus* #2 (2m-P); *Dialictus* #3 (2f-P, 4m-P); *Evylaeus arcatus* (Robertson) (1m-P); *Halictus ligatus* Say (1f-P).
- MEGACHILIDAE: *Heriades carinata* Cresson (3f-P).
- XYLOCOPIDAE: *Ceratina calcarata* Robertson (1f-P; 1f-NP).

DISCUSSION

All three species are facultatively xenogamous, i.e., they have a mixed mating system. The flowers were cross-pollinated, at least in part, when pollinators were active and in their absence some flowers either self-pollinated or were pollinated with self-pollen by thrips. Although we did not test directly the ability of flower visitors to move pollen, the decreased fruit set and pollen deposition on the stigmas of caged and bagged flowers suggests the quite high fruit set of open-pollinated flowers was due in large part to the activity of hymenopteran, dipteran and/or lepidopteran pollinators. Also, Perkins et al. (1975) reported a seed set of 76.3% in male sterile plants of *V. stricta*, which must reflect effective pollination by flower visitors. Further, the likelihood of the flowers of *V. stricta* and *V. hastata* being visited and pollinated was probably increased by their being relatively long lived and producing nectar whether or not they were pollinated.

These species are similar to other facultatively xenogamous species in a number of ways. They are homogamous, self-compatible, at least in part, and produce sufficient nectar to attract pollinators. The pollen-ovule ratios of *V. stricta* and *V. hastata* are equivalent to those of other facultatively xenogamous species, but the P/O of *V. urticifolia* is intermediate between those of typical facultatively xenogamous and facultatively autogamous species (Cruden and Lyon 1989). The fruit set of open-pollinated flowers exceeded 90%, which is typical for facultatively xenogamous species (Cruden and Lyon 1989; Cruden and Westley 1991). The seed set of *V. stricta* (93.3%) was equivalent to the 87.6% reported by Perkins et al. (1975). However, the 91.1% seed set of *V. urticifolia* was much higher than the 66.5% reported by Perkins et al. (1975).

The limited ability to self-pollinate separates these species from most other facultatively xenogamous species that have been studied (Cruden and Lyon 1989). Even within flower pollination by thrips did not yield fruit sets equivalent to those of open-pollinated flowers. In this respect they are similar to *Adenocaulon bicolor* Hook. (Compositae) whose fruit set is high when open-pollinated and quite low when caged (Cruden personal observation).

Pollination Efficiency:

Pollination efficiency, i.e., the percentage of pollen grains produced that reaches the stigmas, is inversely related to mating system with that of xenogamous species being lowest and that of cleistogamous flowers being highest. The pollination efficiency of the three species of *Verbena* (3.8%, 3.0% and 5.0%) was equivalent to that of other facultatively xenogamous species ($\bar{X} = 3.9 \pm 1.8\%$, range $0.25 - 9.72\%$, $n = 5$) and, other than three species of *Asclepias*, only one xenogamous species had a higher efficiency (Cruden 1991). The mean pollination efficiency for xenogamous species other than those in *Asclepias* was $0.435 \pm 0.115\%$ (range $.0025 - 3.1\%$) ($n = 38$). In addition, our estimates of stigmatic pollen loads suggests that open-pollinated flowers of all the species received, on average, more than ten pollen grains per ovule (Table 3), which was undoubtedly sufficient to account for complete seed set (Cruden 1977; Silander and Primack 1978; Snow 1982) and probably assured competition between pollen tubes (Cruden 1991). As was the case in other facultatively xenogamous species (Cruden and Lyon 1989) approximately 98% of the pollen was removed from the flowers of *V. stricta* by flower visitors.

Self-pollination:

At best, the ability of flowers of these species to self-pollinate is limited. The general failure of flowers to self-pollinate was due in large part to the spatial separation of the stigmas and anthers in most individuals, and in *V. stricta* and *V. hastata*, to the curvature of the corolla, which reduced the likelihood of a pollen grain falling from an anther onto the stigma. The fruit set data from the malathion + caged treatment suggest the innate ability of *V. hastata* to self-pollinate is very low. Also, there is the possibility that thrips were responsible for the movement of the small numbers of pollen grains we observed on the stigmas of flowers that had just opened.

The seed set of caged plants in Oklahoma reported by Perkins et al. (1975) is consistent with the conclusion that these species do not regularly self-pollinate. They reported a seed set of 7% in caged plants of *V. stricta*, which was lower than the 32% we observed. This difference may reflect the difference in the separation of the anthers and stigmas in the Oklahoma and Iowa plants, 2 vs. 0.25 mm, respectively. Pollination success of caged plants in the two populations was consistent with the fruit set data. Perkins et al. (1975) observed no pollen on the stigmas of bagged flowers whereas we found a few pollen grains on a few stigmas. Further, the 47.3% seed set by caged plants of *V. urticifolia* in Oklahoma (Perkins et al. 1975) was much lower than expected for a self-pollinating plant and the 40-60% reduction in pollination success we observed in caged plants was consistent with the observed reduction in fruit set.

Pollinators:

The flowers of all the species attracted a diverse array of Hymenoptera, Diptera and/or Lepidoptera that visit the flowers of many plants for nectar and/or pollen (Tables 4-6). Individuals of most of the species that we examined had pollen on the proboscis and elsewhere similar sized insects must be capable of moving pollen between flowers. Because the pollen is carried on the proboscis, male bees and nest parasites, such as *Psithyrus* (Apidae) and *Epeolus* (Anthophoridae), can be effective pollinators. In contrast to our observations, Perkins et al. (1975) found no pollen on the proboscises of any Lepidoptera.

The pollinator guilds we observed were similar to those reported by Robertson (1892) and Perkins et al. (1975) and reflected flower size. The larger flowers of *V. stricta* were visited primarily by medium sized to large bees, various Lepidoptera, and relatively few smaller bees. The failure to observe many flies on the flowers may reflect the dry habitats in which this species lives. The pollinator guild that foraged on the medium sized flowers of *V. hastata* included medium sized solitary bees and flies, and a few larger bees. Late in the season a number of butterflies and moths visited the flowers. Robertson (1892) also reported *Apis, Bombus,* various medium sized solitary bees, and some butterflies. We observed small bees and flies on the small flowers of *V. urticifolia* as did Perkins et al. (1975), who also reported a small butterfly. In contrast, Robertson (1892) reported *Apis, Bombus* and several butterflies in addition to small and medium sized bees.

Although a number of solitary bees visited the flowers of *V. hastata* approximately half of those were individuals of *Calliopsis nebraskensis*. *Verbena* is a major source of pollen for this species (Mitchell, 1960; Krombein et al. 1979). These bees foraged on the flowers from the time of flower opening until late afternoon. Other species were sporadic visitors and were more active on the flowers during the afternoon and most foraged for nectar. The females of *C. nebraskensis* have specialized hairs on the fore legs that are probably used to clean pollen from the proboscis. Two observations support this conclusion. First, we saw nothing to indicate that pollen was removed from the flowers with the fore legs. Second, the proboscises of half (5/10) of the female *Calliopsis* examined bore no obvious pollen, whereas both of the males had pollen on their proboscis as did most of the other solitary bees collected (12/15), as well as 11/14 *Apis* and 9/11 *Bombus*.

The fruit set data suggest that thrips are secondary pollinators of *Verbena,* but might contribute substantively to pollination if the primary pollinators fail to visit the flowers. In both *V. hastata* and *V. stricta* the application of malathion substantially reduced the percentage of flowers setting four seeds and increased the percentage of flowers setting no seeds. The data for *V. stricta* suggest that thrips might account for 50% of the fruit set by flowers not visited by larger insects. The quite low fruit set of caged *V. hastata* may reflect the

experimental design. We used plants that were just coming into flower and thrips may not have been on the plants at the time they were caged, thus reducing the likelihood of thrips reaching the plants and providing an underestimate of thrips pollination.

The high fruit set of two other facultatively xenogamous species are due, at least in part, to the activities of aphids and thrips. Malathion treatments reduced fruit set, i.e., percent achenes/flower, in *Ranunculus sceleratus* L. and *Potentilla rivalis* Nutt. 30-70% (Baker and Cruden unpublished). In these facultatively xenogamous species thrips and/or aphids played an important role in the pollination of flowers that were not visited by pollinators and in small populations they may be the primary pollinators and contribute substantively to a plants fecundity. Elsewhere, only in *Calluna* (Hagerup 1950), *Erica* (Hagerup 1953) and *Arisaema triphyllum* (Rust 1980) have thrips been shown to be primary and/or important pollinators. Thrips were reported as casual or accidental pollinators of *Impatiens* (Valentine 1978) and a few other species (Proctor and Yeo 1972) and may be alternative pollinators of *Phlox* and alfalfa (Faegri and Pijl 1979).

The results we obtained from our cage and malathion + cage treatments revealed ways to improve the protocol. The very low seed set of flowers of *V. hastata* bagged just as the plants started to flower suggested two things. First, plants in full flower should be used in the cage treatment, i.e., plants that are supporting typical insect populations. Second, plants that are just ready to flower should be used in the insecticide + cage treatment. Such plants may not have been colonized or support small populations of insects, which are easily eradicated. Because a single application of malathion failed to eradicate thrips on plants in full flower, we recommend applying the insecticide several times during the course of an experiment. Applying tanglefoot or a similar compound to the base of the inflorescence will keep thrips from crawling up the stem to the flowers. This will be especially effective if inflorescences are bagged.

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REFERENCES

CRUDEN, R.W. 1977. Pollen-ovule ratios: A conservative indicator of breeding systems in flowering plants. Evolution 31:32-46.

CRUDEN, R.W. 1991. Pollen-ovule ratios, pollination efficiency and sex allocation: an examination of alternative hypotheses. In S.L. Buchmann, ed. Experimental studies in pollination and pollinator foraging efficiency. University of Arizona Press, Tucson. (in press)

CRUDEN, R.W. and D.L. LYON. 1989. Facultative xenogamy: Examination of a mixed mating system. pp. 171-207. In J.H. Bock and Y.B. Linhart, eds. The Evolutionary Ecology of Plants. Westview Press, Boulder, CO.

CRUDEN, R.W. and L.C. WESTLEY. 1991. Why is fruit set less than perfect? An examination of the male function hypothesis. Oikos (in press)

FAEGRI, K. and L. VAN DER PIJL. 1979. The principles of pollination ecology. Third revised edition. Pergamon Press, Oxford.

HAGERUP, O. 1950. Thrips pollination of *Calluna*. Biol. Meddel. Kongel. Danske Vidensk. Selsk. 18(4):1-16.

HAGERUP, O. 1953. Thrips pollination of *Erica tetralix*. New Phytol. 52:1-7.

KHO, Y.O. and J. BAER. 1968. Observing pollen tubes by means of fluorescence. Euphytica 17:298-302.

KROMBEIN, K.V., P.D. HURD, Jr., D.R. SMITH, and B.D. BURKS. 1979. Catalog of Hymenoptera in America North of Mexico. Vol. 2. Smithsonian Institution Press, Washington, D.C.

MITCHELL, T.B. 1960. Bees of the Eastern United States. The North Carolina Agricultural Experiment Station, Tech. Bull. 141.

PERKINS, W.E., J.E. ESTES, and R.W. THORP. 1975. Pollination ecology of interspecific hybridization in *Verbena*. Bull. Torr. Bot. Club. 102:194-198.

PROCTOR, M. and P. YEO. 1972. The pollination of flowers. Taplinger Publisher Company, New York.

ROBERTSON, C. 1892. Flowers and insects. Botanical Gazette 17:65-71.

RUST, R.W. 1980. Pollen movement and reproduction in *Arisaema triphyllum*. Bull. Torr. Bot. Club 107:539-542.

RYAN, B.F., B.L. JOINER, and T.A. RYAN, Jr. 1985. Minitab Handbook, Second Edition. Duxbury Press, Boston.

SILANDER, J.A. and R.B. PRIMACK. 1978. Pollination intensity and seed set in the evening primrose (*Oenothera fruticosa*). Amer. Midl. Nat. 100:213-216.

SNOW, A. 1982. Pollination efficiency and potential seed set in *Passiflora vitifolia*. Oecologia (Berlin) 55:231-237.

SOKAL, R.R. and F.J. ROHLF. 1969. Biometry. W.H. Freeman and Company, San Francisco.

VALENTINE, D.H. 1978. The pollination of introduced species, with special reference to the British Isles and the genus *Impatiens*. pp. 117-123. In: A.J. Richards, ed. The pollination of flowers by insects. Linnean Society of London, Academic Press.