Lower Colorado River
Multi-Species Conservation Program

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Balancing Resource Use and Conservation

EFFECTS OF ABIOTIC FACTORS ON INSECT POPULATIONS IN RIPARIAN RESTORATION SITES 2010 ANNUAL REPORT

U.S. DEPARTMENT OF THE INTERIOR
BUREAU OF RECLAMATION

February 2013

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Abstract

Resilin is a structural protein that enables insect cuticle to flex. It has been found in an assortment of structures on a variety of insects. Resilin contains a high nitrogen content and differs from other constituents of insect cuticle by being easy to digest. We estimated amounts of external resilin on insects in 7 orders and 17 families collected at the Beal Restoration Area within Havasu National Wildlife Refuge during 2010. Resilin was detected by photographing insects under long-wave ultraviolet light. Resilin was most abundant on grasshoppers and katydids (Orthoptera) followed by planthoppers and bugs (Hemiptera), dragonflies (Odonata), flies (Diptera), beetles (Coleoptera), and bees and wasps (Hymenoptera). We determined ranked association between the amount of external resilin, relative to body surface area, and percentages of body nitrogen-content measured in the same insects during 2009. Relative amounts of external resilin and nitrogen contents were correlated in the insects examined except for Hymenoptera. Resilin appears to be a primary determinant of nitrogen contents in insects. Habitat constructed for insectivorous wildlife, especially birds, should include marshes to produce dragonflies, insects with high resilin-contents and arrowweed, a host plant for a specific grasshopper that also contains abundant resilin.

Introduction

Eight species of birds (Southwestern Willow Flycatcher, Yellow-Billed Cuckoo, Gilded Flicker, Gila Woodpecker, Vermilion Flycatcher, Bell's Vireo, Sonoran Yellow Warbler, Summer Tanager) and four species of bats (western red bat, western yellow bat, California leaf-nosed bat, pale Townsend's big-eared bat) included in the Lower Colorado River MSCP eat arthropods (spiders and insects). Creating and maintaining habitat for these species will require providing an adequate supply and diversity of arthropods for food. This is especially difficult at several MSCP habitat creation sites being developed, because riparian vegetation is being planted in non-riparian farmland (ie. where water tables are lowered, soil salinities are elevated, and spring flood flows are absent). Growing plants will not by itself guarantee producing populations of spiders and insects diverse and abundant enough to feed and support bird and bat populations. Populations of arthropods are especially limited by concentrations of nitrogen in plants. Different nitrogen contents in spiders and insects also may affect foraging by insectivorous birds. Birds require nitrogen in their food to produce proteins. Spiders or insects especially high in nitrogen may be required as food by adult birds and nestlings.

In 2009, we examined variation in nitrogen content among riparian spiders and insects collected at the Beal Lake MSCP Restoration Site (USBR 2010, Wiesenborn 2011). The objective during 2010 was to examine amounts of resilin, a potential source of nitrogen in insects for riparian birds. Resilin is an elastic protein that allows insect cuticle to flex (Weis-Fogh 1960). It has been found in an assortment of structures in a variety of insects but has not been found in spiders (Andersen and Weis-Fogh 1964). Resilin contains a high nitrogen content (around 19% N) and is easily digested by predators, differing from most insect cuticle. We estimated amounts of external resilin on riparian insects collected at Beal Lake and determined if amounts of resilin are associated with body nitrogen-contents.

Methods

Insects were collected from planted riparian trees and shrubs at the Beal Lake MSCP Restoration Area (Fig. 1) within Havasu National Wildlife Refuge. We swept arthropods from plants and trapped insects with a Malaise trap. Insects were swept from planted cottonwood (*Populus fremontii*), Goodding's black willow (*Salix gooddingii*), honey or screwbean mesquite (*Prosopis* spp.) trees, planted narrow-leaved willow (*Salix exigua*) shrubs, and voluntary arrowweed (*Pluchea sericea*) plants. I also swept arthropods from nearby tamarisk. I collected arthropods on nine dates: 3, 14 & 27 May, 11 & 23 June, 22 July, 4 & 20 August, and 15 September 2010. All sampled plants were in flower or fruit except for cottonwood. The Malaise trap was located in the center of a plot supporting Goodding's black willow and arrowweed. Insects were trapped during 0840-1415 PDT on 3, 14 & 27 May and 22 July 2010. Insects were stored in 70% ethanol.

Fig. 1. Insects were collected from these arrowweed shrubs (foreground) and Goodding's-willow trees (behind) at the Beal Lake MSCP restoration site within Havasu National Wildlife Refuge.

We identified spiders and insects at least to genus and typically to species. Insects were keyed or matched against specimens collected and identified during 2009.

External resilin on insects was detected with ultraviolet light (Weis-Fogh 1960, Andersen and Weis-Fogh 1964). The alkalinity of insects in ethanol was increased to pH 9 with sodium hydroxide. Insects were blotted dry and illuminated with a long-wave (310-390 nm) ultraviolet-lamp that caused resilin, and similar proteins, to fluoresce blue. Insects were photographed with a digital camera using approximately an 8 second exposure time. Blue fluorescence was balanced with reflectance of room lighting by covering the insect with fine-mesh netting. The color intensity of digital photographs (Figs. 2-10) were increased to make blue fluorescence more apparent.

We compared amounts of blue fluorescence with mean nitrogen-contents previously measured in similar insects collected at Beal Lake (USBR 2010, Wiesenborn 2011). In this previous study, nitrogen was measured after being converted to ammonia by digesting insects in acid. Areas of fluorescence, relative to the insect surface-area photographed were ranked. Nitrogen contents of insects, quantified as % N of body dry-mass, also were ranked. Association between relative-area of fluorescence and nitrogen content was tested with Spearman's rank correlation.

Results

Ultraviolet-excited fluorescence was examined on insects in 7 orders, 17 families, and 18 genera (Table 1). All insects were adults except for both nymphs and adults of *Melanoplus herbaceous*.

Table 1. Arthropods collected at the Beal Lake restoration site during 2010 and examined for ultraviolet-excited fluorescence.

Order	Family	Scientific name	Blue fluorescence rank[a]	Mean % N[b]
Odonata	Libellulidae	*Pachydiplax longipennis*	11	12.3
Orthoptera	Acrididae	*Melanoplus longipennis*[c]	15	13.9
	Tettigoniidae	*Scudderia furcata*	14	14.6
Hemiptera	Pentatomidae	*Brochymena sulcata*	13	11.0
	Reduviidae	*Zelus* spp.	12	10.5
	Cixiidae	*Oecleus* sp.	10	10.1
Neuroptera	Chrysopidae	*Chrysoperla* sp.	6	9.1
Coleoptera	Coccinellidae	*Chilocorus cacti*	1	9.8
		Hippodamia convergens	5	6.6
Diptera	Lauxaniidae	*Minettia flaveola*	7	8.1
	Tabanidae	*Tabanus sp.*	2	10.9
	Tachinidae	*Zaira* sp.	8	9.2
	Tephritidae	*Acinia picturata*	9	5.1
Hymenoptera	Andrenidae	*Perdita* sp.	3	9.8
	Formicidae	*Formica* sp.	1	10.9
	Halictidae	*Dieunomia nevadensis*	4	14.1
	Tiphiidae	*Myzinum frontalis*	1	21.2
	Vespidae	*Polistes* sp.	1	14.0

[a]Amounts of blue fluorescence, relative to body surface-area, numbered from lowest to highest.
[b]From USBR 2010, Wiesenborn 2011.
[c]Nymphs.

Nymphs of the grasshopper *Melanoplus herbaceous* produced the most blue-flourescence (Fig. 2). Its pronotum, abdomen, and legs produced a blotchy light-blue fluorescence.

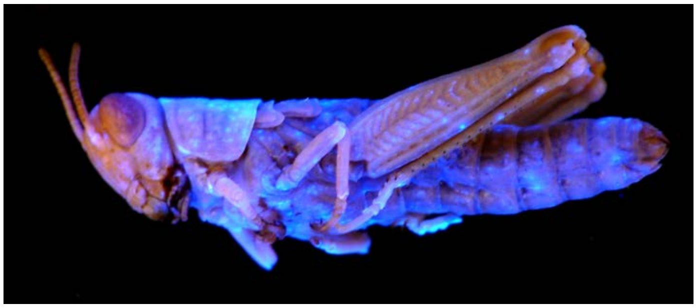

Fig. 2. Blue fluorescence in ultraviolet light on an immature (nymph) grasshopper *Melanoplus herbaceous* (Acrididae) collected from arrowweed.

Patchy fluorescence also was observed on the katydid *Scudderia furcata* (Fig. 3). Fluorescence was seen on its head and abdomen but was not as widely distributed as on *Melanoplus*.

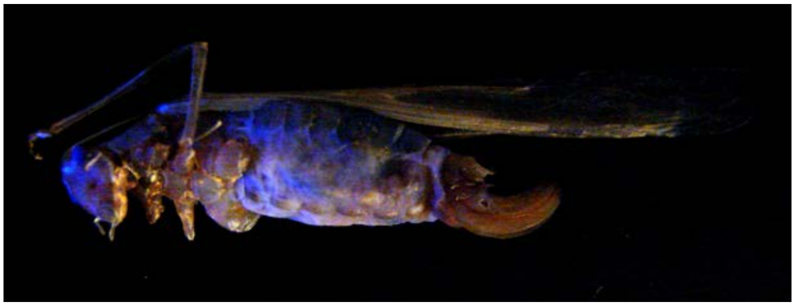

Fig. 3. Fluorescence in ultraviolet light on the katydid *Scudderia furcata* (Tettigoniidae).

The *Zelus* assassin bug also produced blotchy fluorescence (Fig. 4). It fluoresced at the base of its legs and abdomen. Fluorescence was distributed differently on the dragonfly *Pachydiplax longipennis* (Fig. 5). This insect produced a deep, saturated blue that was localized to the hinges beneath the front and hind wings, to the attachment of the base of the abdomen to the thorax, and to sutures (connections) between abdominal segments. Unusual fluorescence occurred on the *Oecleus* planthopper (Fig. 6). Most fluorescence was seen on its eyes and part of its wings.

Fig. 4. Blue fluorescence in ultraviolet light on the ventrum of a *Zelus* assassin bug (Reduviidae).

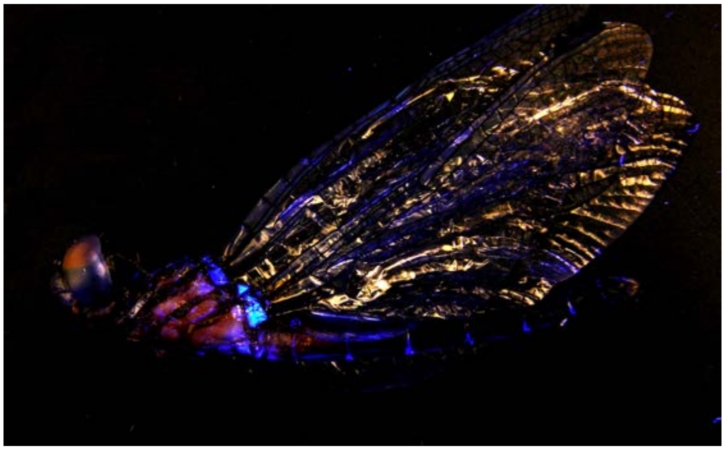

Fig. 5. Blue fluorescence in UV light on the dragonfly *Pachydiplax longipennis* (Libellulidae).

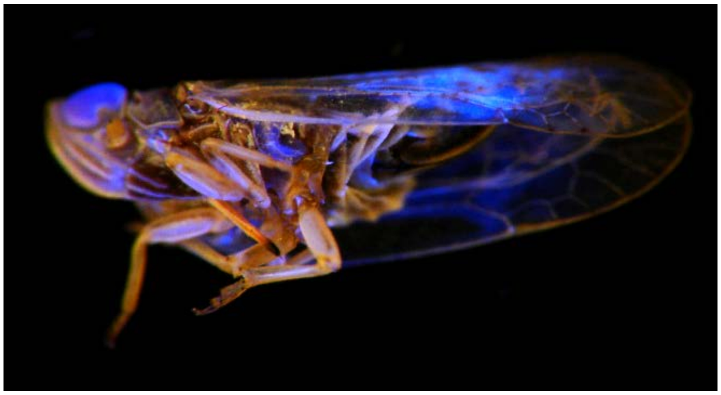

Fig. 6. Blue fluorescence in UV light on an *Oecleus* planthopper (Cixiidae) collected from arrowweed.

The tachinid fly *Zaira* sp. fluoresced only at the base of the leg attachments, below the wing articulations, and at a few sites on the wings (Fig. 7). Tachinid flies develop as parasites within other insects. Those in *Zaira* generally develop within beetle larvae.

Fig. 7. Blue fluorescence in UV light on a parasitic, *Zaira* fly (Tachinidae) caught with a Malaise trap.

Fluorescence was absent or near-absent on Hymenoptera. As examples, the *Polistes* paper wasp (Fig. 8) and the *Formica* ant did not exhibit blue fluorescence under ultraviolet light.

Fig. 8. A paper wasp, *Polistes* (Vespidae), photographed under ultraviolet and visible light.

Fig. 9. A *Formica* ant (Formicidae), photographed under ultraviolet and visible light.

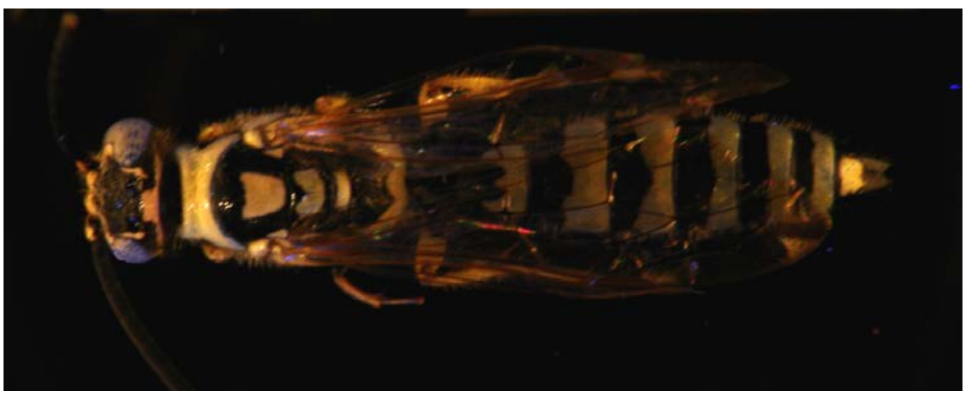

Fig. 10. A tiphiid wasp (Tiphiidae) collected from arrowweed and photographed under ultraviolet and visible light.

Amounts of external fluorescence, relative to the surface area of the insect photographed, generally increased as percentages of body nitrogen increased except in the five Hymenoptera genera examined (Fig. 11).

When all seven orders of insects were examined, ranked amounts of fluorescence and nitrogen contents were not correlated (r = -0.09; n = 17; t = 0.35; P = 0.36). When Hymenoptera (bees and wasps) were excluded, ranked amounts of fluorescence and nitrogen contents were positively associated (r = 0.62; n = 12; t = 2.47; P = 0.017). Relative amount of fluorescence and body nitrogen-content were positively associated in Odonata (dragonflies), Orthoptera (grasshoppers and katydids), Hemiptera (stink bugs, assassin bugs, planthoppers) Neuroptera (lacewings), Coleoptera (beetles), and Diptera (flies) but not in Hymenoptera.

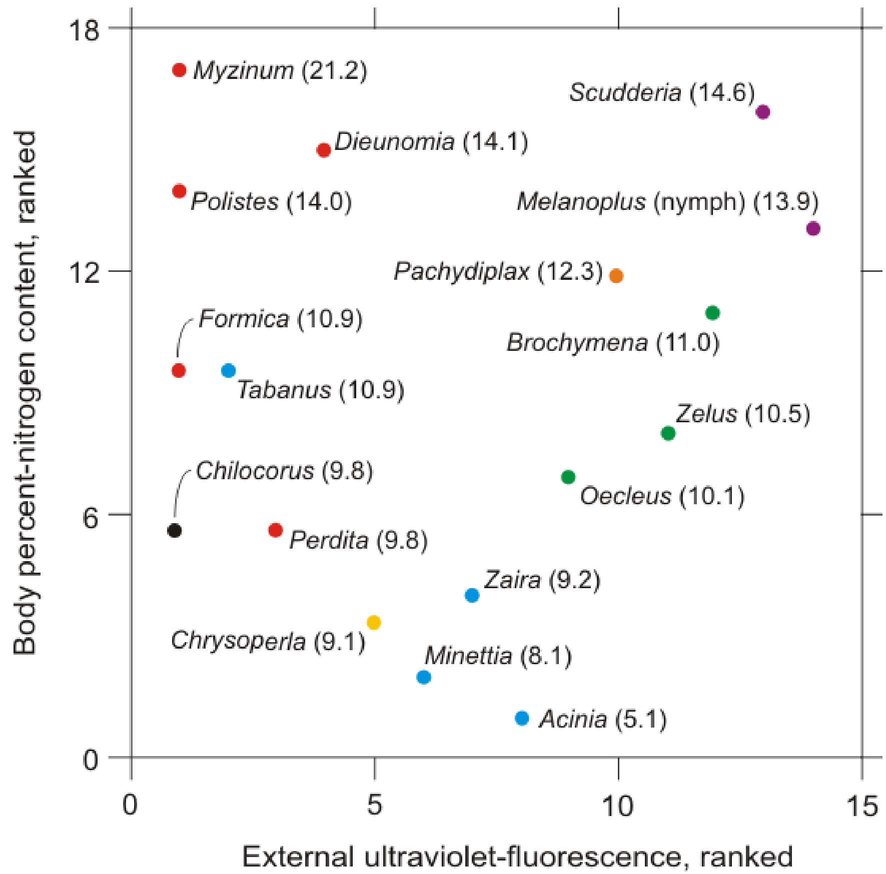

Fig. 11. Genera of insects plotted in relation to ranked, body percent-nitrogen content (in parentheses) and ranked amount of external ultraviolet-excited fluorescence, relative to body surface-area. Colors of points represent insect orders (see Table 1, above).

Discussion

Illuminating insects with long-wave ultraviolet light enabled observing fluorescence of resilin. The saturated, blue fluorescence characteristic of resilin was especially evident beneath the wings on the *Pachydiplax* dragonfly. These fluorescent areas are the wing-hinge ligaments, structures on dragonflies first discovered to contain resilin (Weis-Fogh 1960, Andersen and Weis-Fogh 1964). Dragonflies are relatively-primitive insects with direct flight-muscles, muscles that attach with ligaments directly to the base of the wings. The elasticity of resilin allows the flapping motion of the wings.

Blue fluorescence also was observed beneath the wing on the *Zaira* tachinid fly. Less fluorescence was seen compared with the dragonfly. Wing hinges are smaller on flies than on dragonflies, because flies employ indirect muscles to move their wings. Instead of attaching to the wings themselves, the muscles attach to the sides of the body. The lateral contraction and expansion of the body (thorax) causes the wings to move up and down.

Blue fluorescence was distributed differently on the *Oecleus* planthopper. Most fluorescence was observed on its wings and compound eyes. Other studies have found resilin within the membrane of insects wings and on compound eyes. Resilin has been suggested to enable elasticity of wings, improving flight, and transparency of the outer covering of compound eyes.

Fluorescence on the *Melanoplus* grasshopper, *Scudderia* katydid, and *Zelus* assassin bug differed from the other insects examined. Rather than being isolated to a particular structure, fluorescence was instead dispersed in a blotchy pattern over most of the body. Fluorescence also was a lighter blue and somewhat grainy. The source of this fluorescence is unclear. Neville (1975) suggested that this light-blue fluorescence was due to a protein similar to resilin.

The absence or near-absence of fluorescence on Hymenoptera indicates that resilin is absent, rare, or not externally-visible on bees and wasps. Hymenoptera are the most-advanced of the insects examined. Thus it appears that resilin is more abundant on primitive insects, such as dragonflies, and less abundant on advanced insects such as bees and wasps.

Excluding Hymenoptera, insects with greater amounts of external resilin contained higher body-concentrations of nitrogen. Resilin therefore appears to be a significant source of nitrogen to insectivorous birds. Insects with greater amounts of resilin provide insectivorous birds with an abundant and digestible source of nitrogen. Although nitrogen contents were high in Hymenoptera, the nitrogen may not be in a form that is digestible by birds.

These results emphasisize the importance of establishing a diverse source of insects for insectivorous birds at MSCP restoration sites. Of particular interest are dragonflies. Remains of dragonflies were found in fecal samples collected from southwestern willow flycatchers at Topock Marsh during 2004 (Wiesenborn and Heydon 2007). The abundant resilin and high nitrogen-contents of dragonflies, combined with their large size, suggest dragonflies are a significant source of food for flycatchers and other insectivorous birds. The dragonflies at Topock Marsh likely developed as immatures within the marsh. Aquatic habitat, producing dragonflies and other aquatic insects may be an important component of restored riparian habitat. Aquatic habitat is absent at Palo Verde Ecological Reserve and at Cibola Valley Conservation Area. The Laguna Division restoration site now being planned will benefit from the aquatic habitat that the project will incorporate.

Another insect providing large amounts of resilin and high concentrations of nitrogen are the *M. herbaceous* grasshoppers. These were the only grasshoppers collected at the Beal Lake restoration site, and their only host plant is arrowweed (Strohecker et al. 1968). Arrowweed is a native plant, despite its common name, and supports an assortment of insects and spiders. The arrowweed at Beal Lake, volunteering mainly along dirt roads, exploits the site's irrigation and therefore is especially productive as a source of insects.

Habitat restored as part of the MSCP should strive to create a variety of habitat, especially aquatic, and a variety of plants to support a diverse prey base of insects and spiders. Diverse insects provide an assortment of nutrients needed by insectivorous birds and other wildlife.

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