**History of Creeping Indigo Toxicity in Florida**

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In South Florida, a mysterious and often fatal condition of adult horses and livestock had begun to be recognized in the 1970s.1 Affected horses were dull, apathetic and uncoordinated; some had convulsions, ulcers of the tongue, and whitening or streaking of the corneas of the eyes.  Most of them died within weeks. The problem was seen most commonly in the late summer or fall.

Beginning in 1973, a University of Miami botanist, Julia Morton, began to walk the properties on which these deaths were occurring. She noticed an abundance of wild indigo plants (*Indigofera suffruticosa*) and initially supposed that these were the culprits. By the 1980s, however, Dr. Morton had investigated many more incidents and had become convinced that the condition was actually caused by a different indigo herb, *creeping indigo* (also known as trailing indigo; *Indigofera spicata*), that had widely invaded local pastures and was being enthusiastically eaten by horses. In 1987, she decided to present her findings at a local meeting of concerned equine veterinarians and horseowners. Dr. Morton explained that creeping indigo had first been imported into the United States in 1933, and established at (embarrassingly) the University of Florida in Gainesville, so that its supposed utility as livestock forage and ground cover could be studied. When it was found that rabbits died when they grazed the experimental plots, the project was quickly abandoned!2 The neglected but apparently hardy plants escaped containment, invaded locally, and supposedly spread inexorably southward over the subsequent decades; they now can be found growing along the sidewalks of Key West. Dr. Morton also noted reports from Queensland of Birdsville Indigo Poisoning, an almost identical nervous system disease of horses grazing another *Indigofera* species, *I. linnae*.3

The presentation was well attended but Dr. Morton’s creeping indigo theory was not well accepted, with many attendees holding fast to the notion that toxic chemicals sprayed on trees in local fruit groves were the problem – thus the name “Grove poisoning.” By the early 1990s, even the doubters had acknowledged the obvious and, although feeding trials have thankfully not been performed in horses to formally establish cause and effect, it is now generally accepted that creeping indigo causes the neurologic syndrome previously known as Grove poisoning.

Over the last 10 years, we have seen similar small outbreaks of the disease in horses and in donkeys in central and north-central parts of the state, especially in areas north of Tampa and around Brooksville. This extension in geographic range of the syndrome appears to correspond to increasing abundance of creeping indigo in the central and north central parts of the state. The origins of this recent wave of invasion by creeping indigo are unclear. It is interesting to hypothesize possible roles for climate change or for a separate introduction or emergence of a new, more invasive strain. This is explored more fully below. In any event, creeping indigo has become a very common plant around Alachua and Seminole counties and can be found growing along the edges of many pathways, including many at the University of Florida. Even more threateningly, it has become the dominant herb in some pastures in the Ocala area.

**Creeping Indigo**

The Indigofereae are a small tribe of often very attractive, pink to red-flowered shrubs and herbs. The largest genus is *Indigofera* which is pantropical, with 75% of the ~750 species restricted to Africa–Madagascar, and the rest in the Sino-Himalayan region, Australia, Mexico, and Brazil.4 Because of their complex chemistry, *Indigofera* contain many toxic and medicinally used species. These toxic chemicals have evolved as a defensive response to predation by herbivores and pathogens.5 Other *Indigofera* are economically important indigo dye-producing and pasture legume species that occupy an extremely wide range of different habitats. *Indigofera spicata* is native to East Africa and Madagascar, Indonesia, and the Philippines. It was valued as a cover crop in coffee estates in Africa and was introduced into India, Java, Malaya, and the Philippines, both as ornamental ground cover and a cover crop for tea, rubber, oil palm, and sisal plantations. It has also been introduced into Australia, several pacific islands, and the Caribbean.

Surprisingly, there is still some confusion as to the identity of the creeping indigo plants originally described by Dr. Morton. At the time she published her work, *I. spicata/I. hendecaphylla* were considered by some to be synonymous and referred to as *I. spicata* complex.6 By 2006, there was a school of thinking among Australian botanists that these were actually different species and Dr. Morton had in fact described *I. hendecaphylla.*7 This seemingly obscure point has currently excited some debate among local botanists and a definitive conclusions to the story is surely at hand; however, for now, multiple exemplars of the plant harvested around Gainesville have been identified by the University of Florida Herbarium as *I. spicata* on the basis of the latter’s relatively smaller leaves, flowers, and pods. If these identifications are correct, it suggests that there are *two* closely related species of creeping indigo in Florida with their respective origins somewhat obscure. Both *I. hendecaphylla* and *I. spicata* have been connected with equine and livestock deaths, although such an association remains to be confirmed for *I. spicata* in Florida. Thus it will be important to collect plants from all future outbreaks and have them identified by the UF Herbarium.

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| Table 1. Comparison of characteristics of two closely related creeping indigo species, *I. hendecaphylla* and *I. spicata.*  |
| **Feature** | ***I. hendecaphylla*** | ***I. spicata*** |
| Posture | Prostrate spreading perennial | Prostrate spreading perennial |
| Leaves | Pinnate, 7-10 leaflets, 20-55 mm, | Pinnate, 5-7 leaflets, alternate, 12-40 mm, |
| Leaflets | Obovate to elliptical, 5-16 × 3-7 mm, dull green | Obovate, 4-11 × 3-8 mm, dull green |
| Inflorescences | Red, 120-150 mm | Pink to orange-red, 30-65 mm |
| Pods | Descending, 22-27 mm, brown | Descending, 10-18 mm, yellow-brown |

Both plants are prostrate to sub-erect with branched runners fanning out in all directions from the crown of a white, slender, tapering taproot firmly set in the earth and descending nearly a meter (Figures 1-3).7,8 The stems are pale-green to yellow, tough, and thickly set with alternate, pinnate, clover-like leaves that are 1-5 cm long. The slender, tubular flowers are brick-red to pink (mostly pink around Gainesville). The most characteristic and identifiable feature, the needle-like, stiff, sharp-tipped seed pods, 1-3 cm long, are borne profusely in dense, downward-pointing clusters. The plant, perennial in tropical and subtropical climates, is killed back in winter in central and north-central Florida but sprouts from the root in spring.

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Figure 1. *Indigo spicata* specimens obtained at the University of Florida and authenticated at the UF Herbarium

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Figure 2. *Indigo spicata* showing typical pink flowers and clusters of pods (upper part of image) growing at University of Florida, Gainesville, FL.

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Figure 3. *Indigo* sp. Dense thatch of creeping indigo comprising >40% of a bahiagrass pasture in Ocala, FL. Note the abundant pods and evidence of grazing by horses in the pasture.

**Signs of Creeping Indigo Toxicity1,3,6,9,10**

Consumption of 10 pounds *I. linneae* daily for 3 weeks is sufficient to cause the very similar Birdsville disease.3,9 Both neurologic and non-neurologic signs are seen.

*Non-neurologic signs.* There may be weight loss, inappetence, high heart and respiratory rates, labored breathing, high temperature (a rare finding), hypersalivation (ptyalism) or foaming from the mouth, dehydration, pale mucous membranes, feed retention in the cheeks (quidding), halitosis, watery discharge from the eyes (epiphora; Figure 4) and squinting (blepharospasm), light sensitivity, corneal opacity, corneal ulceration and  neovascularization, severe ulceration of the tongue and gums (Figure 5), and prominent digital pulses without other signs of laminitis.

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Figure 4. 11-year-old mixed breed gelding with creeping indigo toxicity from near Brooksville, FL. Note the dull appearance, staining with ocular discharge below the eye, and flaccid lower lip. On close inspection there was also marked edema of the right cornea and blepharospasm or the right eyelids.

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Figure 5. 5-year-old Paso Fino gelding with creeping indigo toxicity from near Homestead FL. Note the extensive ulcerations of the gums above the upper incisors. This horse’s tongue was also severely ulcerated.

*Neurologic signs.* Often, an early sign is a change in personality – affected horses at first seem quieter and less energetic than usual. Degrees of obtundation (depression) ranging from mild lethargy to recumbency and loss of consciousness may be seen as the condition progresses over days to weeks. Head carriage is low and there may be episodes of standing sleep-like activity (narcolepsy), head-pressing into corners, or compulsive walking around the inside of a stall or paddock. Some affected horses have been seen with their heads tilted to one side and their necks and bodies twisted in the same direction indicating involvement of the balance centers (vestibular system) of the brain. These signs may be accompanied by rhythmic blinking and jerking eye movements (nystagmus). The blink response to hand gestures toward the eyes (menace response) is frequently absent or reduced although constriction of the pupils to bright light is usually retained. The muzzle and lips may hang flaccidly. In retrospect, it is often clear that an abnormal gait has been seen developing over the preceding several days, characterized by incoordination and weakness in all limbs, with unpredictable crossing of pairs of limbs, interference between hooves, buckling of joints during weight-bearing, a “crab-like” gait and abnormal posturing at rest. Some affected horses develop a bizarre goose-stepping gait in their front legs. Most horses that continue to consume the plant eventually become cast on their sides and are unable to rise. They either become unconscious or develop convulsions which may become generalized and severe before death or euthanasia after days in recumbency.

*Laboratory Findings.*Abnormal findings on routine hemograms and plasma chemistry panels are mild, non-specific, and unhelpful in making the diagnosis. Typically, there is low-normal or low total white blood cell count with lymphopenia and mild electrolyte derangement that may include hyponatremia, hypophosphatemia, hypomagnesemia and metabolic acidosis. Aspartate aminotransferase and creatine kinase activities often are slightly to moderately above reference range.

*Necropsy Findings*. Both gross and histologic examinations of horses poisoned by creeping indigo are most notable for the *lack* of diagnostic findings. It has been shown in experimental animals that subacute and chronic toxicity by 3-NPA, the creeping indigo toxin that causes neurologic signs, does not cause changes evident on light microscopy, although Brazilian investigators documented changes by electron microscopy of the mitochondria of horses that had consumed *I. lespedezioides*.10 Horses may show mild liver pathology, evident histologically as periacinar necrosis, infiltration of lymphocytes and haemosiderin-laden macrophages in periacinar regions and vacuolation and swelling of surviving hepatocytes 6,11.

**Toxins**

Over the last decade, there has been resolution of the previous confusion about the roles of the two putative toxins of creeping indigo, 3-nitropropionate (3-NPA) and indospicine. It is now clear that 3-NPA causes the largely irreversible neurologic signs described above while indospicine causes the corneal edema, ulcerations, and other non-neurologic signs.6 Each of these toxins is described briefly:

*3-nitropropionate.*3-NPA is a highly toxic compound produced by the plant primarily as defense against destruction by herbivores.11,12 This nitrotoxin is by no means unique to *Indigofera* but is produced as anti-herbivore defense by a vast array of plants and fungi. Poisoning associated with 3-NPA therefore occurs quite commonly in a variety of settings and the mechanism is well understood. The toxin is a potent and irreversible inhibitor of mitochondrial succinate dehydrogenase, a key enzyme in transforming glucose and oxygen into useable energy.12 Nerve cells are extremely vulnerable to energy deprivation, thus accounting for the early and prominent neurologic signs seen with all types of 3-NPA toxicity.  3-NPA accounts for 0.24 to 1.5% of the dry matter of creeping indigo. Because it is metabolized quickly, it is unlikely to be found in the serum of affected animals.

*Indospicine*. Indospicine13 is a non-protein amino acid. It is toxic to the liver because of antagonism to the essential amino acid arginine, with which it competes. One of its principal toxic actions is inhibition of nitric oxide synthase, an action likely associated with the development of corneal edema and ulceration of mucous membranes. Although horses are relatively resistant to the liver damaging effects of this toxin, it persists in the tissues of horses dying or killed with the disease, and these tissues are potentially toxic if fed to dogs. Indospicine accounts for 0.1 to 0.5% of the dry matter of creeping indigo and it can be detected in the serum of affected animals.

**Treatment and prevention**

Horses that are quickly removed from the offending plants may recover completely, but more often there are persistent gait abnormalities. There is no effective treatment. Early investigations into the14 prevention and treatment of Birdsville disease in Australia proposed the use of arginine-rich protein sources such as peanut meal (4.3% arginine) and gelatin (8.0% arginine).9 The lack of significant liver lesions in horses because of their relative resistance to indospicine, together with the likelihood that the neurological disease results from 3-NPA poisoning, suggests that arginine alone would have little benefit in the treatment of nervous signs although nonneurologic signs may respond. Thiamine was also suggested as an effective treatment for nitro toxicity in ruminants but others showed this treatment to be ineffective.6 The fact that 3-NPA neurodegeneration is used as an induction model for Huntington’s disease research is testament to the current futility of all treatments.15 Management of affected horses should include their removal from the source, confinement to prevent any injuries and non-specific supportive therapy. It was previously suggested that livestock poisonings by *I. spicata* can be prevented by keeping the proportion of this plant below 25% of the total forage available but recent evidence does not support this view.14 The best means for preventing poisoning is to stop access by horses to paddocks where creeping indigo is present or to remove plants by physical means or herbicide application.

Although there is no herbicide commercially recommended for eradication of creeping indigo, the University of Florida Pasture Weed Identification and Control page at the IFAS Extension EDIS website (<https://edis.ifas.ufl.edu/topic_pasture_weeds>), suggests spraying either of two herbicides containing *aminopyralid*: Milestone (Dow AgroChemicals) at 5 fl oz per acre or GrazonNext HL (Dow AgroChemical) at 24 fl oz per acre. Retreatment the following year will likely be necessary. Dead plants retain toxicity and must be removed and disposed of. Manure from animals that graze herbicide-treated pastures should not be composted. Also, any grass clippings removed from these treated pastures should not be composted. Be sure to follow all label directions when using pesticides!

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