Accumulation of nitrate by annual goldeneye and showy goldeneye

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Abstract

Annual goldeneye [*Viguiera annua* (M.E. Jones) Blake] and showy goldeneye [*Viguiera multiflora* (Nutt.) Blake], grown in the greenhouse and from the field, were analyzed for cyanide, soluble oxalates, alkaloids, nitro compounds, and nitrate. Both species were investigated for their ability to accumulate toxic levels of nitrate when grown in nutrient solution, or in soils fertilized with ammonium nitrate.

Both species tested negative for cyanide, soluble oxalates, nitro compounds, and alkaloids. The plants accumulated toxic levels of nitrate, calculated as KNO_3, when grown in nutrient solutions (4 to 5%), greenhouse soil fertilized with ammonium nitrate at 220 kg N/ha (3 to 4%), or from plots fertilized with ammonium nitrate in the field at 66 to 220 kg N/ha (2 to 3%). Annual goldeneye collected near a water tank and salt lick on a New Mexico ranch contained 4.7% nitrate but plants collected at other sites contained only 0.05 to 0.7% nitrate. Goldeneye in the field may accumulate toxic levels of nitrate if rooted on soils heavily contaminated by animal excreta around water tanks, ponds, salt licks, and along frequently used trails. Livestock losses from goldeneye can be reduced or prevented by avoiding the plants and by treating affected animals with methylene blue.

Key Words: *Viguiera annua*, *Viguiera multiflora*, poisonous plants, chemical analysis, nitrogen

One hundred fifty species of *Viguiera* (Asteraceae) are indigenous to the Western Hemisphere, principally from western North America to central South America (Hitchcock et al. 1955). One species, annual goldeneye [*Viguiera annua* (M.E. Jones) Blake], has been reported poisonous to cattle (Kingsbury 1964, Norris and Valentine 1954). The poisonous principle was not identified, but toxic signs described for cattle suggested either cyanide or nitrate as the toxic compound.

Annual goldeneye is found from Texas, Arizona, and New Mexico to southern Utah (Martin and Hutchins 1980). This erect, slender annual has linear to linear-lanceolate leaves, mostly 3 to 7 cm long and 1.5 to 3 mm wide. The 6 to 8 mm-wide floral disk is surrounded by about 12, brilliant yellow rays 5 to 12 mm long. In New Mexico, where livestock losses have been reported, the plant grows primarily on south and west facing slopes of hills and mesas, from north central to southern and western counties, and from 1,200 to 2,200 m elevation. The plant flowers in late summer and early fall.

Showy goldeneye [*Viguiera multiflora* (Nutt.) Blake], for which no losses have been reported, occurs over a broader range from Montana and Wyoming to New Mexico and Arizona (Hitchcock et al. 1955). This perennial species has lanceolate to ovate-lanceolate leaves, and the disk and ray flowers are larger than those of annual goldeneye. This species was included in this investigation to determine if the toxic properties were present in both annual and perennial species of goldeneye.

Losses from annual goldeneye are sporadic and tend to occur only in years when environmental conditions favor prolific germination and abundant stands of the plant. During years when goldeneye is abundant, the plant may account for 40 to 50% of the foliar cover on some sites. Plants tend to be most dangerous if timely autumn rains produce a fresh flush of growth in September and October. Severe cattle losses from annual goldeneye occurred over 80,000 to 100,000 ha of southwestern New Mexico in 1949 (personal communication, Jupe Means, Buckhorn, NM). Goldeneye was unusually abundant throughout the region. In 1983, goldeneye was again abundant, but losses were less severe. Losses occurred in the fall when goldeneye was in flower and animals were being trailed. Toxic signs in poisoned animals included dyspnea, trembling, weakness, and collapse. In nonfatal cases, animals recovered after removal from the goldeneye and rest.

The present study was initiated to identify the toxic compound in goldeneye, determine the conditions under which the plant becomes poisonous, and to recommend measures to prevent poisoning and treat affected livestock.

Materials and Methods

Mature seed of annual goldeneye was collected 5 November 1986, on the H-Y Ranch near Buckhorn, Grand County, New Mexico. Seed of showy goldeneye was collected 20 September 1986, near Tony Grove Lake in the mountains east of Logan, Utah. The seed was cleaned, placed in sealed bottles, and held at 1° C until March 1987. The seed of both species failed to germinate in late March even after rinsing with running water for 48 hours. Germination was achieved by rinsing the seed under running water for 24 hours, then excising the embryos, rinsing the embryos for 48 hours under running water, then placing them on moist filter paper in petri dishes. Once a root of 5 mm had developed, the seedlings were transferred to a 5:1 vermiculite-soil mixture and grown to the 4-leaf stage.

Goldeneye seedlings of both species in the 4-leaf stage were transferred on 4 May to 4-liter polyethylene pots that contained Hoagland's nutrient solution (Hoagland and Arnon 1950). The solution contained 210 mg N/1 as $Ca(NO_3)_2$ and KNO_3. Chelated iron, however, was used as the source of iron. Ten pots were used per species and 2 plants were planted in each pot. The plants were grown under a 16-hour photoperiod. Fluorescent lights placed 3 feet above the plants augmented normal daylength. A maximum temperature of 26° C was maintained by evaporative cooling and exhaust fans. The plants were grown for 42 days to a height of 25 to 30 cm. Three mature leaves were then removed at random from all plants of each species, washed, dried, pooled, ground, and analyzed for percent nitrate. The plants were harvested 14 days later and samples of leaves and stems of both species analyzed for percent nitrate. Showy goldeneye was in flower at the second harvest; annual goldeneye was vegetative.

In a second experiment, both species of goldeneye were grown in 15 cm styrofoam pots in a 1:3 mixture of sand and mountain soil until approximately 25 cm high. One plant was placed in each pot, and the pots were in full sunlight on a greenhouse bench. One-half of the plants were given nitrogen as ammonium nitrate in amounts

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equivalent to 220 kg N/ha dissolved in 100 ml of water and poured once onto the the soil surface. Soil moisture was maintained near field capacity for the duration of the study. Three replications of 6 plants each were used. Mature leaves from 6 plants in each replication were collected 2, 6, and 10 days after treatment, pooled, washed, dried at 50° C for 24 hr in a forced air oven, ground, and analyzed for percent nitrate.

Nine, 1-m² plots were established near Tony Grove Lake in the Cache National Forest in June 1987 when showy goldeneye was 10 to 15 cm high. Ammonium nitrate was broadcast by hand to the soil in 3 replications at 66, 132, and 220 kg N/ha on 25 June. Leaves and stems were sampled at random from each of the plots on 25 June, 14 July, and 23 July, pooled by replication and treatment, and analyzed for percent nitrate.

Whole plant samples of annual goldeneye were collected on the H-Y Ranch near Buckhorn, New Mexico, on 3 August 1987. Plants were vegetative and 20 to 24 cm high. Plants were collected at random from 7 sites, including 1 near the Wicker Flats water tank and salt lick, by roads along which cattle were trailed, and at range sites remote from trails and water. The plants were pooled by site, dried, ground, and analyzed for percent nitrate. No treatments were involved. Four, 1-m² plots with goldeneye were established in a pasture near ranch headquarters. Ammonium nitrate was applied to the soil at a rate equivalent to 220 kg N/ha on 3 August. The fertilizer was dissolved into the soil with a sprinkler and also by rain which fell during the first night after application. Leaf and stem samples were collected 18 and 27 August, dried, and sent to Logan, Utah, for analysis.

Whole plant samples of both species collected in the greenhouse and field during vegetative growth were pooled and analyzed for cyanide (Anonymous 1980), soluble oxalates (Dye 1956), alkaloids (Cundiff and Markunas 1955), and nitro compounds (Williams and Norris 1969) by methods previously described. Analyses for nitrate nitrogen were conducted by the Soils Testing Laboratory at Utah State University. Nitrates are expressed as percent KNO_3, on a dry weight basis.

A split-split plot analysis of variance was used in which the whole plot treatments were rates of ammonium nitrate, the first split was the plant part, and the second split was time to account for repeated measures.

Results and Discussion

Annual and showy goldeneye from the field and greenhouse tested negative for cyanide, nitro compounds, soluble oxalates, and alkaloids. Potentially lethal levels of nitrate were found in plants grown in nutrient solutions, in soil to which ammonium nitrate was added, and near the water tank on the H-Y Ranch. A nitrate level of 0.5 to 1.5% (as KNO_3) in plants is considered toxic to livestock, and a concentration of 1.5% and above is potentially lethal (Kingsbury 1964).

Both species of goldeneye contained toxic levels of nitrate in the leaves at all collection dates when grown in nutrient solution. Sampled after 6 weeks of growth in nutrient solution, leaves of annual and showy goldeneye contained 5.4% and 4.5% nitrate, respectively. When plants were harvested 2 weeks later, nitrate concentration in both leaves and stems of annual goldeneye was 3.5%; and in showy goldeneye leaves and stems, nitrate levels were 2.3 and 2.6%, respectively.

A significant increase in nitrate levels occurred in leaves of soil-grown goldeneye when ammonium nitrate was added at 220 kg N/ha. Within 2 days, the nitrate level increased over 6 times in annual goldeneye and over 5 times in showy goldeneye (Table 1). Six days after treatment, the concentration of nitrate in showy goldeneye was more than 8 times greater than the control. High nitrate levels persisted for at least 10 days.

Table 1. Effect of fertilization with ammonium nitrate in the greenhouse on nitrate concentration in leaves of annual and showy goldeneye grown in soil.

Species	Treatment	Rate (kg N/ha)	Date of collection: July 18[1]	July 20	July 24	July 28
			% Nitrate[2]			
Annual goldeneye	Control	0	0.6	0.6	0.5	0.6
Annual goldeneye	NH_4NO_3	220	0.6	3.9	3.1	3.5
Showy goldeneye	Control	0	0.6	0.5	0.5	0.5
Showy goldeneye	NH_4NO_3	220	0.7	2.6	4.3	4.1

LSD (0.05) = 0.29 for differences between treatments for a fixed date.
LSD (0.05) = 0.23 for differences between treatments for different dates.

[1]Date of treatment.
[2]Calculated as KNO_3.

The nitrate concentration of showy goldeneye fertilized in the field at Tony Grove Lake increased 5 to 22 times over the 4-week test period (Table 2). Nitrate levels in the leaves and stems of the unfertilized controls were very low. Nitrate levels in leaves and stems of plants from treated plots were significantly higher than the controls at the 2 July collections. Significantly more nitrate was found in the stems than in the leaves of treated plants. Thus, toxic levels of nitrate accumulate in both perennial and annual goldeneye if sufficient nitrogen is available.

Table 2. Effect of fertilization with ammonium nitrate on nitrate concentration in showy goldeneye in the field.

Treatment	Rate (kg N/ha)	Plant part	Date of collection: 25 June[1]	14 July	23 July
			% Nitrate[2]		
Control	0	Leaves	0.05	0.05	0.1
Control	0	Stems	0.1	0.1	0.1
Ammonium nitrate	66	Leaves	0.05	1.3	0.7
Ammonium nitrate	66	Stems	0.1	2.1	2.2
Ammonium nitrate	132	Leaves	0.1	0.5	0.6
Ammonium nitrate	132	Stems	0.1	2.1	1.8
Ammonium nitrate	220	Leaves	0.05	0.6	0.5
Ammonium nitrate	220	Stems	0.1	1.5	2.0

LSD (0.05) = 0.28 for differences between leaves and stems, same treatment and date.
LSD (0.05) = 0.28 for differences between control and treated leaves; control and treated stems, for same date.

[1]Date of treatment.
[2]Calculated as KNO_3.

Annual goldeneye, fertilized with ammonium nitrate adjacent to ranch headquarters, accumulated nitrate at toxic levels (Table 3). Plants from unfertilized plots contained moderate levels of nitrate. A single horse kept in this pasture apparently contributed sufficient nitrogen through excreta to elevate nitrate levels in control plants. As observed in showy goldeneye, nitrate levels were higher in the stems than in the leaves.

Nitrate levels in annual goldeneye collected on the H-Y Range ranged from an innocuous 0.05% to a highly toxic 4.7% (Table 4). The plants that contained 4.7% nitrate were collected near a water tank and salt lick on an area heavily contaminated with animal excreta. This area apparently provided the plants with an abundance of available nitrogen, and this resulted in plants high in

Table 3. Effect of fertilization with ammonium nitrate on nitrate concentration in annual goldeneye in the field.

			Date of collection		
Treatment	Rate (kg N/ha)	Plant part	3 Aug.[1]	18 Aug.	27 Aug.
			% Nitrate[2]		
Control	0	Leaves	0.4	0.4	0.5
Control	0	Stems	0.9	0.7	0.7
Ammonium nitrate	220	Leaves	0.5	1.9	1.0
Ammonium nitrate	220	Stems	0.8	2.9	2.0

LSD (0.05) = 0.48 for differences between leaves and stems, same treatment and date.
LSD (0.05) = 0.44 for differences between control and treated leaves; control and treated stems, same date.

[1]Date of treatment.
[2]Calculated as KNO_3.

nitrate. The majority of cattle poisonings on this ranch in 1983 occurred near this area. Plants with considerably less nitrate (0.3 to 0.7%) were collected beside a road along which cattle had been trailed in the fall. Some excreta was deposited on these sites, although in much smaller amounts than at the Wicker Flats water tank. The 2 sites at which plants contained only negligible amounts of 0.05% nitrate were away from the road on sites less commonly frequented by cattle.

Annual and showy goldeneye accumulate nitrate in toxic quantities if nitrogen is available in higher than normal concentrations. Plants may accumulate toxic levels of nitrate in the field if they grow on sites where livestock tend to congregate such as watering areas, salt licks, or areas through which animals are trailed. The nitrogen supplied by excreta concentrated in these areas may be accumulated by goldeneye as nitrate. Greenhouse tests indicated that nontoxic goldeneye could accumulate toxic levels of nitrate within 2 days after fertilization with ammonium nitrate. Thus, fall rains that produce a fresh flush of growth may also result in toxic goldeneye if plants are rooted in areas where animal excreta provides abundant, available nitrogen. On less fertile soils, the plants may never be toxic.

Annual goldeneye is an increaser on disturbed sites, particularly near ponds and salt licks where density of grass has been reduced by overgrazing or trampling. On some sites on the H-Y Ranch where tobosagrass (*Hilaria mutica* Benth.) was the dominant grass, goldeneye comprised 40 to 50% of the foliar cover. Density of goldeneye, however, bears no relationship to toxicity or threat to livestock unless sufficient nitrogen is available for nitrate accumulation.

Precipitation data for Cliff, New Mexico, the weather station near the H-Y Ranch, were examined from 1948 through 1984. Average yearly precipitation for these years was 34.7 cm, with most occurring July through September and again in December and January. Total precipitation from 1 December 1948 through 30 November 1949 was 38.8 cm; for 1 December 1982 through 30 November 1983, 56.0 cm. Approximately 80% of these amounts fell from December through February and August through September. Winter precipitation for both periods was nearly twice normal.

Table 4. Nitrate analyses of annual goldeneye collected 3 August 1987 on the H-Y Ranch near Buckhorn, New Mexico.

Plant part	% Nitrate[1]	Site
Whole plant	4.7	Wicker Flat water tank and salt lick
Whole plant	0.7	Bear Mountain, adjacent to road
Whole plant	0.5	One-half mile south of antelope tank
Whole plant	0.3	One-fourth mile southwest of Wicker Flat
Whole plant	0.3	Three-fourth mile west of Bathtub Canyon
Whole plant	0.05	Bear Mountain, west facing slope 500 ft below road
Whole plant	0.05	Bear Mountain, 300 ft above road

[1]Calculated as KNO_3.

Livestock losses can be reduced by controlling annual goldeneye in areas where animals congregate and where abundant animal excreta would provide a source of nitrogen to the plant. However, no herbicides have been recommended for control of annual goldeneye.

Nitrate from plants is quickly converted to nitrite in the rumen. Once absorbed into the blood, nitrite complexes with ferrous hemoglobin to form methemoglobin, which cannot transport oxygen. Death from asphyxiation occurs when methemoglobin levels are sufficiently high so that the blood no longer carries enough oxygen to sustain life. Animals affected by nitrate poisoning should be kept as quiet as possible to keep oxygen demand low. Comatose animals may survive methemoglobin levels of 85%, whereas active animals may die if methemoglobin levels are 50 to 60% (Kingsbury 1964). Affected livestock may be successfully treated by intravenous injection of methylene blue, an effective antimethemoglobinemic compound.

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