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Viability of weed seeds in feed pellet processing[1]

S. DENNIS CASH, DAVID L. ZAMORA, and ANDREW W. LENSSEN

Authors are Extension Agronomist, Department of Plant, Soil and Environmental Sciences, Montana State University, Bozeman, Mont.; independent consultant, Boise, Ida.; and Research Assistant Professor, Department of Entomology, Montana State University, Bozeman, Mont., respectively. Published with the approval of the Director of the Mont. Agr. Exp. Sta. as Journal Series J-5075.

Abstract:

Federal and state agencies in several western states now require the use of noxious weed-free or noxious weed seed-free forage to hinder the spread of noxious weeds. Forage can be certified as noxious weed-free through state administered programs. Processed feeds such as pellets or cubes made from noncertified hay and uncleaned grain are some of the forage products that may be potential sources of weed infestations. This study was conducted to determine levels of weed seed contamination in alfalfa hay/grain feed pellets manufactured with commercial-grade equipment. Seeds of whitetop [*Cardaria draba* (L.) Hand.], spotted knapweed (*Centaurea maculosa* Lam.), Canada thistle [*Cirsium arvense* (L.) Scop.], leafy spurge (*Euphorbia esula* L.), and common yellow sweetclover [*Melilotus officinalis* (L.) Lam.) were added in known quantities to alfalfa/grass mixed bay and to barley. The hay was ground in a hammermill through a screen with 7.9-mm diameter perforations, and the barley was ground to pass through a 2.4-mm screen. In a second experiment, uncertified 'Ladak 65' alfalfa (*Medicago saliva* L.) seeds were ground with alfalfa/grass mixed hay in a hammermill and extruded through a pellet die before being ground In another hammermill with barley grain followed by extrusion through a pellet die. The Montana Department of Agriculture collected pelleted feed from various manufacturers in the state during 1993 and 1994 to estimate potential weed contamination frequency. Grinding of weed seeds with alfalfa hay or barley grain reduced emergence by 98 to 100%. Grinding and pelleting reduced emergence of alfalfa seed by over 99%. Weed seedlings emerged from 11% of random feed pellet samples collected from Montana manufacturers. Rigorous processing such as oc-

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curs when manufacturing hay/grain pellets reduces the risk or disseminating weed seeds from pelleted feed.

Keywords:

Medicago saliva, *Hordeum vulgare*, *Cardaria draba*, *Centaurea maculosa*, *Cirsium arvense*, *Euphorbia esula*, noxious weed seed-free forage.

The USDA Forest Service is attempting to limit the spread of nonnative plants into national forests by requiring that outfitters, recreationists, and agencies use noxious weed-seed free feed for pack and saddle stock. Western states are cooperating with the Forest Service and other agencies by establishing field inspection/certification programs to certify weed seed-free forage. Alfalfa (*Medicago sativa* L.) hay, grass hay, alfalfa/grass hay, cereal-grain hay, straw, and forage pellets and cubes are examples of products which can be certified as weed seed-free feeds.

Pelleted feed is manufactured by reducing particle size through grinding and compacting the particles into pellets. The grinding process varies among feed pellet manufacturers, but most grinding is done with hammermills (Pfost 1976). Particle size is determined primarily by screen perforation diameter, but also by hammer width, the clearance between hammer tip and screen, and hammer tip speed (35 to 127 in sec^{-2}).

Pellet manufacturers typically use a tub grinder, a large capacity hammermill with a perforated screen, to grind bales weighing from 450 to 900 kg. Grinding is completed in a smaller hammermill equipped with a screen that has hole diameters of less than 1.27 cm followed by pelleting and storage. Complete-ration feed pellets are processed by mixing grain and hay pellets, followed by grinding and final pelleting. Grain is ground to pass through a screen with hole diameters that are smaller than those used for hay. Hay is ground at least twice, and grain is typically ground only once before extrusion through the pellet die.

Steam is often injected into the conditioning chamber for lubrication and to liberate natural oils. Steam partially gelatinizes starches in seeds (Pfost 1976), however it is unknown if the combined effects of heat and steam pressure are sufficient to destroy viable seeds. After extrusion through the die, the pellets are bagged or conveyed to storage bins.

Few studies have examined the effects of pellet manufacturing on seed viability. Lyon and Rush (1993) found that jointed goatgrass (*Aegilops cylindrica* Host) survived grinding through a hammermill or roller mill. Zamora and Olivarez (1994) found that seeds of timothy (*Phleum pratense* L.), alfalfa (*Medicago sativa* L.), spotted knapweed (*Centaurea maculosa* Lam.), and sulfur cinquefoil (*Potentilla recta* L.) mixed with barley (*Hordeum vulgare* L.) grain can survive grinding in hammermills and extrusion through die-and-roller pellet mills. They conducted these tests with a small hammermill that may not have simulated conditions in a large commercial feed producing plant. They also did not determine the combined effects of grinding and pelleting on mortality of seeds added to hay. The objective of this study was to determine if commercial feed manufacturing equipment destroys all weed seeds. Weed seeds were introduced into alfalfa/grass mixed hay and barley grain for processing into feed pellets. Samples of pelleted feed from manufacturers throughout Montana were tested also for identification and viability of seed contaminants.

Materials and methods

Seeds of whitetop [*Cardaria draba* (L.) Hand.], spotted knapweed, Canada thistle [*Cirsium arvense* (L.) Scop.], and leafy spurge. (*Euphorbia esula* L.) were collected during the summer of 1994. Uncertified common yellow sweetclover [*Melilotus officinalis* (L.) Lam.] and 'Ladak 65' alfalfa seed were purchased locally. Percentage germination and emergence of weed seeds before grinding or pelleting were determined in concurrent laboratory and greenhouse tests. Standard germination tests were conducted by placing 100 seeds of each species on blotters in each of 4 boxes (replicates), and maintained in a controlled environment of 12 hours at 25°C followed by 12 hours at 15°C. A second set of germination boxes were maintained for 7 days in the dark at 5°C, prior to placement in the germination chamber to determine if stratification would significantly improve germination. Germinated seeds were counted daily up to 28 days.

Emergence tests in the greenhouse were conducted by planting all species in each of 4 flats (replicates) with a 10% (weight/weight) mixture of alfalfa hay meal or barley meal with Sunshine™ media potting mix. Four additional flats without added weed seeds were included as controls. After planting, the flats were subirrigated, treated with a 0.1% (weight/volume) solution of thiophanate methyl fungicide, and covered with newspapers for 4 days. Alternating day/night temperatures in the greenhouse were 24 and 18°C, respectively. Ambient light was extended to 16 hours with high pressure, sodium vapor lamps. Emergence counts were taken at 15-day intervals to 60 days.

The germination and emergence data were transformed (square root of the arcsine), and differences among species within a test were evaluated by the Friedman rank test (Lehman, 1975) in the Minitab Version 11 software (State College, Penn.). The Friedman test was chosen as the appropriate tool to handle nonparametric data cases. Means were separated by a multiple pair-wise testing statistic at the $p = 0.05$ level of significance. Across germination and emergence tests, simple analyses of variance were computed within each species on the transformed data. Actual means are presented, and mean separations at the $p = 0.05$ level of significance based on the transformed data are included where appropriate.

The feed grinding and pelleting experiments were conducted at Western States Industries, Inc. in Choteau, Mont. The grinding equipment specifications are described in Table 1. The perforation diameters in the screens and pellet die sizes used in these tests are typical for those reported in a 1994 survey by the Montana Feed Association (Cash, unpublished data). New screens were installed in both the hay and grain grinding hammermills prior to the tests.

Alfalfa hay meal and barley meal

Six 45-kg bales of alfalfa/grass mixed hay were each inoculated with a mixture of seeds of whitetop, spotted knapweed, Canada thistle, leafy spurge, and yellow sweetclover. The seed mixture added to each bale had a known quantity of germinable seed of each species, based on availability. Each bale (replicate) passed through a Bale Buster™ (which has no screen) then through a Teco™ hammermill equipped with a 7.9-mm screen. Each bale was ground, and the hay-weed seed meal was mechanically mixed and bagged separately for analysis. The tub grinder and the pellet extruder were not used in this experiment because of the large batch quantities required (454 kg minimum) for each replicate, which would have required more weed seeds than were available.

Six 11.3-kg lots (replicates) of barley were each inoculated with a mixture of seeds of the 5 weed species listed above. The 6 grain and weed composites were then ground in a Jacobsen™ hammermill equipped with a screen perforated with 2.4-mm diameter holes (Table 1), and bagged separately.

Table 1. Description of equipment used to grind alfalfa hay and barley for pelleting trials.

Equipment specifications	Bale Buster™	Teco™ hammermill	Jacobsen™ hammermill
Power (kW)	56	149	56
Rotation (rev min^{-1})	1,750	1,750	3,600
Hammer speed (m s^{-1})	46	83	86
Hammer size (cm)	1.3 × 5.1 × 25.4	0.6 × 5.7 × 20.3	0.6 × 5.4 × 12.1
Hammer-to-screen clearance (mm)	no screen	1.6	1.6
Screen size, l × w (cm)	no screen	91 × 112	71 × 56
Screen perforation diameter[1] (mm)	no screen	7.9	2.4

[1]New screens were installed for this study.

Greenhouse emergence tests were conducted as previously described to estimate seed survival. Six 100-g subsamples were planted for all replicate hay or barley meal samples. Emerged seedlings were counted at 15-day intervals for 45 days. Emergence data were standardized by expressing the emergence percentage of each species based on its laboratory germination. These percentage data were transformed (square root of the arcsine), and analyzed by the Friedman rank test, and means were separated at the $p = 0.05$ level of significance.

Feed pellets

In a separate test, the entire feed pellet process was evaluated. Alfalfa hay pellets were formed from a mixture of 6 bales of alfalfa/grass mixed hay plus 48.5 kg of 'Ladak 65' alfalfa seed. Before grinding, a hay probe was used to extract 28-g hay samples from each of the 6 bales as controls. The hay bales (224 kg total) were ground through the Bale Buster™ and Teco™ hammermill equipped with a screen perforated with 7.9-mm holes. Alfalfa seeds were added directly to the Teco™ hammermill without going through the Bale Buster™. The ground hay and seed were conveyed to the feed chamber of a pellet mill (California Pellet Mill Company, Crawfordsville, Ind.). The pellet mill was a dual speed, 112 kW, die-and-roller pelletizer equipped with a 6.4-cm thick die (5.1-cm effective thickness) perforated with 6.4-mm diameter holes. Steam was injected into the conditioning chamber of the pellet mill at 276 kPa. The pellets were cooled and then reground with 51 kg of barley grain in the Jacobsen™ hammermill equipped with a screen perforated with 2.4-mm diameter holes. Reground hay pellets were thoroughly mixed with ground barley (80/20, weight/weight) and extruded through the pellet mill under the same conditions described for the alfalfa pellets.

Six samples were collected at each step in the manufacturing process: 1) unground hay prior to processing, 2) hay and seed meal after grinding in the Teco™ hammermill, 3) pelleted hay plus seed, 4) meal from reground hay pellets plus ground barley, and 5) final pellets made from the reground hay pellets plus barley. Due to the batch sizes required to utilize all steps in the pelleting process, and lack of identity control within the mill, this experiment was replicated in time. By timing the flow between the respective hammermills and the pellet extruder, it was possible to take 6 repeated samples to provide comparisons among treatments 2 through 5 above. These samples were tested for weed seedling emergence in the greenhouse using the same procedures and growing conditions as previously described.

State pellet samples

The Plant Industries Division of the Montana Department of Agriculture collected 70 samples of various pelletized feed manufactured in 1993 or 1994. The Montana Department of Agriculture also collected background information on the feeds and methods for making the pellets (data not reported). Unreplicated, 100-g samples from each pellet sample were planted in the greenhouse as previously described. Seedlings were identified and counted between 45 and 180 days after planting to survey for the potential number and types of weed seeds present in commercial pellets.

Results and discussion

Alfalfa hay meal and barley meal

The effects of feed grinding on weed seed mortality were estimated in this experiment by greenhouse emergence tests. Hand separations and counting of seeds in hay or grain meal is very time-consuming, and may not accurately predict the viability of collected seeds. Laboratory germination in a standard 28-day test ranged from 21% for whitetop to 96% for yellow sweetclover (Table 2). Across all species, stratification by prechilling did not significantly increase laboratory germination, therefore meal and pellet samples were not stratified. Initial emergence tests were evaluated up to 60 days (data not reported), however due to very little increase from 45 days, all further trials were terminated at 45 days. Percent germination of the tested species in the laboratory was higher than percent emergence in the greenhouse in hay or barley meal. Most of this could have been due to the difference in seedling survival in the greenhouse compared to the ideal laboratory conditions. The alfalfa hay meal appeared to have a suppressive effect on emergence of all species except leafy spurge and whitetop. Interestingly, whitetop had slightly higher emergence in hay meal than in barley meal. Most of these differences in greenhouse emergence were not statistically significant ($p = 0.05$), and likely were insufficient to alter our interpretation of the data. All species had adequate 45-day greenhouse emergence potentials for use as a rapid assay to measure the effects of the grinding and pelleting experiments.

Table 2. Seed germination in standard laboratory evaluations and greenhouse emergence of 6 species after mixing with uninfested alfalfa hay meal or barley meal.

Species	Laboratory germination (28-day)				Greenhouse emergence (45-day)				Mean
	No prechill		Prechill		Alfalfa hay meal		Barley meal		
	(%)				(%)				(%)
Yellow sweetclover	96.0 A	a[1]	93.5 A	a	67.5 A	b	81.8 A	ab	94.7 A
Alfalfa	88.5 AB	a	90.8 A	a	73.8 A	a	79.8 A	a	83.1 AB
Spotted knapweed	84.3 B	ab	89.8 A	a	38.0 AB	b	51.5 AB	b	65.9 BC
Canada thistle	53.3 BC	a	60.0 B	a	15.0 C	b	27.5 B	ab	38.9 CD
Leafy spurge	25.8 C	ab	32.5 B	a	19.3 C	b	21.3 B	ab	24.7 D
Whitetop	21.3 C	ab	35.5 B	a	20.5 BC	ab	5.0 B	b	20.6 D
Mean	61.5	ab	67.0	a	39.0	b	44.5	b	

[1]Means within a column followed by the same upper case letter or within a row followed by the same lower case letter are not significantly different at $p = 0.05$.

In the grinding tests, 24,593 and 67,612 germinable weed seeds kg^{-1} mere added to hay and barley, respectively (Table 3). Recovery of germinable weed seeds in the hay meal ranged from 0% for leafy spurge to 1.3% for whitetop. In barley meal, recovery ranged from 0% for leafy spurge to 0.3% for yellow sweetclover. Leafy spurge seed was wider but not longer or heavier than the other species (Table 4), which may have contributed to seed coat damage and its failure to emerge after being ground. Other factors that contribute to seed damage differences among species are starch and fiber content (Pfost, 1976). Seeds with higher starch contents are easier to grind (Pfost, 1976). The barley and alfalfa meals were not visually examined for intact leafy spurge seed that may have been dormant or dead because of a damaged seed coat.

Fewer seedlings of whitetop, spotted knapweed, Canada thistle, and yellow sweetclover emerged after seeds were ground through a screen with 2.4-mm diameter perforations than through a screen with 7.9-mm diameter perforations (Table 3). Although direct statistical comparisons were not appropriate, the differences in mortality range from 3 to

27 times higher for the smaller screen. Zamora and Olivarez (1994) also found that quantity of seeds recovered and percent germination of recovered seeds declined as the diameter of screen perforations in a hammermill decreased. Lyon and Rush (1993) reported that fewer jointed goatgrass caryopses germinated after grinding through a screen perforated with 4-mm diameter holes compared to caryopses ground through a screen with 4.8-mm diameter holes.

Table 3. Weed seeds added to alfalfa hay or barley and subsequent greenhouse emergence in meal samples after grinding.

Species	Germinable seed added to:		Emergence (45-day)	
	Alfalfa Hay	Barley	Alfalfa Meal (7.9 mm)	Barley Meal (2.4 mm)
	(N kg^{-1})		(%)	
Spotted knapweed	2,229[1]	3,744	0.81 AB[2]	0.03 B
Canada thistle	728	2,187	1.83 A	0.19 AB
Leafy spurge	761	2,281	0.00 B	0.00 B
Whitetop	8,265	24,823	1.29 A	0.28 A
Yellow sweetclover	12,610	34,577	0.93 AB	0.30 A

[1]Number of germinable seed based on laboratory germination percentage for each species.
[2]Means within a column followed by the same letter are not statistically different at p = 0.05.

In addition to the weed seeds added in these tests, other plant species emerged from the alfalfa and barley meal. Forb species emerging from the meal were prostrate vervain (*Verbena bracteata* Lag. & Rodr.), blue mustard [*Chorispora tenella* (Pall.)], pinnate tansymustard [*Descurainia pinnata* (Walt.)], catnip (*Nepeta cataria* L.), and creeping woodsorrel (*Oxalis corniculata* L.). Grass species emerging from the meal were creeping bentgrass (*Agrostis stolonifera* L.), cheat (*Bromus secalinus* L.), orchardgrass (*Dactylis glomerata L.*), quackgrass [*Elytrigia repens* (L.) Nevski], barley, red fescue (*Festuca rubra* L.), and timothy (*Phleum pratense* L.). These species could have been contaminants of the deliberately-added weed seeds or may have been present in the hay bales or barley.

Table 4. Physical characteristics of seeds of 6 species used in pellet trials.

Test species	1,000-Seed weight $\bar{x}$ (SE)	Length $\bar{x}$ (SE)	Width $\bar{x}$ (SE)
	(g)	(mm)	(mm)
Whitetop	0.61 (0.03)	2.45 (0.05)	1.40 (0.02)
Spotted knapweed	1.71 (0.03)	2.94 (0.05)	1. 12 (0.06)
Canada thistle	0.70 (0.03)	2.91 (0.05)	0.86 (0.03)
Leafy spurge	1.79 (0.03)	2.40 (0.05)	1.54 (0.03)
Alfalfa	2.10 (0.05)	2.45 (0.05)	1.41 (0.03)
Yellow sweetclover	2.06 (0.03)	2.19 (0.05)	1.48 (0.03)
N	3	10	10

Feed pellets

Through the entire process, only 0.01% of the alfalfa seeds that were initially ground and pelleted with alfalfa hay were recovered in final feed pellets (Table 5). Each step in the production of feed pellets resulted in progressively fewer seedlings emerging 45 days after planting in the greenhouse. Progressive grinding and pelleting resulted in stepwise differences in alfalfa seed mortality. Pelletizing of the hay and barley meals resulted in 92 and 86% reductions in emergence, respectively. Zamora and Olivarez (1994) also showed that the combination of grinding and extrusion through a pellet die decreased alfalfa seed viability more than either process alone. The current study showed that even after being ground and extruded through a pellet die twice, a small number of seed still survived.

A higher percentage (4.06%) of alfalfa seeds emerged after a single grinding (Table 5) than from the average of all weed species (0.97%) (Table 3). Both were ground through the Teco™ hammermill with the same size screen. Seed coats of alfalfa may be harder than the seed coats of the other species allowing more seed to survive grinding. However, alfalfa seed did not go through the Bale Buster™ which also may have destroyed some seed. Three weed species (cheat, blue mustard, and tansy mustard) emerged from the core samples of the hay bales prior to grinding and pelleting.

Temperature of the pellets immediately after extrusion was approximately 56.7°C, and the pellets were exposed to this temperature briefly. Zamora and Olivarez (1994) measured pellet die temperatures of 57.2 and 62.2°C for hay-grain and grain pellets, respectively. After extrusion, pellets are quickly cooled to about 8°C above ambient temperature (Robinson 1976). Exposure to 56.7°C probably has little affect on alfalfa seed viability. In other studies, viability of unimbibed seeds of 8 weed species tested was unaffected after 6 hours exposure to 70°C (Egley 1990). Standard methods used by the Animal and Plant Health Inspection Service for complete sterilization (seed death) require autoclaving for 15 minutes at 69 kilopascal with an approximate temperature of 115°C or injecting steam into loose material until all parts reach 100°C (personal communication, Klag). The Animal and Plant Health Inspection Service also found that exposing various federal noxious weed seeds to 100°C for 15 minutes does not kill all seeds (unpublished data, Westbrooks, Whiteville, N.C.).

Table 5. Greenhouse emergence of alfalfa seed added to hay and processed into feed pellets.

Component	Emergence (45-day)
	—— (%) ——
1. Hay core	0.00[1]
2. Meal from mixing 224 kg hay + 48.5 kg alfalfa seed, hammermill (7.9 mm)	4.06 A[2]
3. Pellet from hay and seed meal	0.50 B
4. Meal from hay pellets mixed with barley, hammermill (2.4 mm)	0.07 C
5. Pellet from reground hay and seed plus barley	0.01 D

[1]No alfalfa emerged; however, seedlings of cheat, blue mustard, and tansy mustard emerged at rates of 9,178, and 464 kg^{-1} hay, respectively.

[2]Emergence based on 88.8% laboratory germination and known quantities of ingredients added.

State pellet samples

Eleven percent of the pellet samples collected from Montana feed manufacturers had 1 or more weed seedlings emerge (data not reported). Six samples had a white clover (*Trifolium repens* L.) seedling, 1 had a wild mustard [*Brassica kaber* (D.C.) Wheeler] seedling, and 1 sample had 6 plants from 4 species wild mustard, common lambsquarters (*Chenopodium album* L.), catnip, and prostrate vervain. Contaminated samples had weed seed densities ranging from 10 to 60 kg^{-1} pellets. Most of the samples with the lowest level of contamination were finished products. The sample with 60 seeds kg^{-1} of pellets was intended for further grinding and pelleting with hay. Only 21 of the 70 samples tested were manufactured specifically for use as horse feed. Weeds emerged from only 2 of these 21 samples, with each having 1 white clover seedling. White clover was also 1 of 2 species (4 seedlings total) that emerged during a similar test of 80 pellet samples conducted in Montana in 1991 (unpublished data, Fay). It is unknown if the white clover came from the pellets or from the greenhouse potting soil where it can be a common contaminant.

The background information collected with the state pellet samples was insufficient to make any correlations between weed seed occurrence and pellet components or manufacturing processes. Details of the manufacturing procedure for specific batches of pellets may be nonexistent because of the variable conditions required to pellet feed. The American Feed Manufacturer's Association has said that every run should be approached from the viewpoint of a new formulation (MacBain 1967).

Grain and alfalfa hay often contain weed seeds that are incorporated into feed pellets. The number of weed seeds in grain or alfalfa hay must be known to predict if the pellets will contain viable seeds. Zamora and Olivarez (1994) concluded that because most grain lots used for feed pellets are not cleaned it is highly probable that feed pellets manufactured from it would be contaminated. In a drillbox survey, 85% of all the samples of uncleaned grain (spring wheat, winter wheat, and spring barley) contained weed seeds (Dewey *et al*. 1985). In that study, the average number of weed seeds in uncleaned grain was about 550 seeds kg^{-1}.

Most weed seeds in uncleaned grain will be destroyed during the pelleting process. Average weed emergence in grain was 0.16% after grinding to pass through a screen with hole diameter of 2.4 mm (Table 3). If percent emergence is reduced by another 84 to 92% after extrusion through a pellet die as occurred for alfalfa seeds (Table 5), 0.02% of all weed seeds in grain would still be viable. There would be only 0.02 viable weed seeds kg^{-1} of horse feed pellets consisting of 20% grain by weight if 0.02% survived pelleting.

Conclusions

Seeds in unprocessed hay consumed by livestock before entering national forests may likely spread more exotic species than seed in feed pellets. The pellet manufacturing system tested in this study destroyed over 99% of viable weed seeds added to alfalfa hay or grain. Based on this study and previous work, it appears that most seed mortality is caused in feed grinding to pass through progressively smaller perforations. Heat and steam pressure at the pellet extruder reduced alfalfa seed survival by over 86%. Our results document that pellet processing can greatly reduce the germination of weed seeds. Livestock can excrete viable seeds of many species consumed in contaminated feed for several days after consumption (Janzen 1981, Thill *et al*. 1986). The introduction of weed seeds into national forests consumed by livestock before entering national forests is likely impossible to stop. There is a very low probability that weed seeds will survive grinding and pelleting followed by mastication and digestion. It appears that feed pellets formulated from noncertified alfalfa hay or grain pose only a slight risk of contaminating public lands, as long as these products are manufactured with similar processes and ground through similar sized screens as were used in this study. To fully understand the potential of feed to spread weed seeds to national forests, data are needed on the species and amount of weed seed in certified, weed-free forage, noncertified forage, and cubed hay.

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