Session 8

Mare Reproductive Loss Syndrome Monitoring Programs for 2002

Chairperson: Dr. M. Scott Smith, Dean, College of Agriculture, University of Kentucky

Overview of the Mare Reproductive Loss Syndrome Monitoring Program for 2002

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DURING THE SPRING OF 2001, THE KENTUCKY HORSE industry experienced a severe and sudden outbreak of early fetal loss (EFL) and late fetal loss (LFL), birth of weak foals, and cases of pericarditis and unilateral uveitis. Barren and maiden mares, early foaling mares that were bred in February and March, and late foaling mares bred in April and May of all breeds were affected. These conditions were collectively termed Mare Reproductive Loss Syndrome (MRLS), whose total economic loss to Kentucky exceeded $300 million.

The sudden and widespread occurrence of MRLS indicated that the causal agent was probably environmental or pasture related. In spite of intensive sampling, assays of several pasture parameters failed to show correlations with incidence of MRLS symptoms. Due to the sudden onset, it was impossible to sample pastures before fetal losses, making subsequent pasture assays difficult to interpret. A lack of baseline information about several pasture parameters also hindered interpretation. Farm visits during mid-May discovered a strong correlation of MRLS symptoms with the presence of black cherry trees (BCT), *Prunus serotina*, and exposure of mares to the eastern tent caterpillar (ETC), M*alacosoma americanum*. Other common pasture characteristics associated with MRLS included large amounts of white clover (*Trifolium repens* L.). Tall fescue (*Festuca arundinacea* Schreb.) was noted in several but not all MRLS fields.

Over the late summer and fall of 2001, several theories were advanced as to the potential causal agent for MRLS. These included cyanide content of ETC, cyanide content of white clover, ergopeptide alkaloids of tall fescue, mineral imbalances of pasture, nitrate content of pasture, and fungal mycotoxins associated with Fusarium and other fungi. In addition, late frost events during 2001 seemed to be coincidental with onset of MRLS. The incidence of poison hemlock (*Conium maculatum*) was also advanced as a possible cause for MRLS.

A monitoring plan was developed to follow these and other pasture and environmental parameters during 2002. This plan was developed with input from university scientists, private consultants to the equine industry, veterinarians, and farm managers. The goal of the monitoring program was to define "normal" levels of several pasture parameters thought to be related to MRLS and to monitor their impact on susceptible populations of horses (barren or maiden mares bred early or mares foaling after May 1).

Materials and Methods

In the fall of 2001, the University of Kentucky College of Agriculture outlined a monitoring system to follow pasture and weather parameters in Central Kentucky for 2002. From February 21 to June 30, 2002, data were collected from 13 Central Kentucky farms (12 horse farms and one hay production farm) on a biweekly basis to determine their potential relationship or correlation with MRLS. Monitored horse farms were selected to include both high-risk and low-risk Thoroughbred and Standardbred farms in Bourbon, Fayette, Jessamine, and Woodford counties. Risk level was determined by MRLS losses in 2001. Fields for each horse farm included barren or maiden mares and late foaling mares (April-May foaling). The monitoring program followed specific groups of mares over the duration of the sampling period and therefore would sample several pastures over the course of the season. Fields for the hay production farm included an alfalfa (*Medicago sativa*) and timothy (*Phleum pratense*) field. Data collected on mares included mare identity, breeding dates, reproductive status, clinical signs, and if any MRLS symptoms were experienced in 2001. A subset of horse farms collected blood and urine samples on a voluntary basis. These samples were stored for future analysis.

Additional fields from monitored farms were also sampled if MRLS symptoms occurred. Other farms (six) outside the monitoring program were monitored on a request basis after MRLS symptoms occurred. Field management history was recorded for all fields monitored and included fertilizer, lime, and herbicide application and rates, mowing frequency, and height. The presence or absence and management of BCT and ETC were also recorded.

Visits were made every two weeks for the majority of the season and weekly during late April and early May. At each visit, forage and soil samples were taken to characterize each pasture for loline and ergopeptides alkaloids (both in tall fescue alone and in the composite pasture), nitrate, cyanide (white clover only), fungal mycotoxins, soil yeasts and molds, forage minerals, and soil minerals. In pastures where BCT were present, samples were taken for pasture ergopeptides, fungal

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mycotoxins, and soil yeasts and molds in and out of proximity to BCT.

Forage samples were clipped manually and placed in paper bags except for white clover, which was placed in plastic bags. Samples for loline, ergopeptide, and forage mineral and nitrate analysis were placed daily in a forced air oven and dried at 50°C. White clover samples were kept refrigerated until analysis. Samples for fungal mycotoxin (forage tissue) and microbial population characterization (soil) were placed in paper bags and refrigerated until analysis.

Loline and ergopeptide analysis was done by gas chromatography (GC) and HPLC techniques, respectively. Fungal mycotoxins were measured initially by TLC and HPLC techniques and in the latter part of the sampling period by ELISA methods. Yeast and mold counts were determined by plate count methods.

Forage nitrate was analyzed with colorimetric techniques using cadmium reduction, and minerals were analyzed by atomic absorption methods. Cyanide levels in white clover were determined by standard EPA colorimetric procedures. Individual farms also collected rainfall as well as daily minimum and maximum temperatures from March through June.

Additional samples representing the composite pasture forage were taken from each pasture during each cycle and frozen to provide tissue for future analyses as needed based on future research or observations. For fields with BCT, two samples were taken: one from areas close to BCT and one from the remainder of the field.

Additional samples collected and stored or observations on a request basis included cyanide level of BCT leaves and flowers; ETC larvae, pupae, frass, and tents; and poisonous plants, including members of the Apiaceae family (*Conium maculatum*, poison hemlock). All samples were delivered to appropriate labs or frozen and stored on a daily basis.

The monitoring program included the formation of an oversight committee composed of University of Kentucky scientists and veterinarians, veterinarians in private practice and with the Kentucky Department of Agriculture, as well as horse farm managers. The role of the oversight committee was to oversee and advise the University of Kentucky as to any needed modifications in the monitoring plan and to provide feedback from affected and interested groups.

Results

From February to June, 75 fields were monitored as a part of the routine sampling program with additional fields added as required for follow-up of EFL or LFL. Routine soil samples were taken from each field at the start of the program. Loline and ergopeptide assays were conducted on 631 samples (260 from tall fescue alone and 371 representing total pasture species). Fungal mycotoxins and microbial counts were assayed on 402 and 400 samples, respectively. Nitrate-N and cyanide levels were measured on 246 and 244 samples, respectively.

Half (six) of the 12 horse farms on the routine monitoring program experienced MRLS losses (32 EFL and 9 LFL). These losses were associated with 13 different fields and occurred from April 25 to June 13. Of those fields with losses, only three did not have BCT either in or near them. All follow-up visits to additional farms with MRLS losses (from veterinary referrals) found potential for exposure to ETC.

Ergopeptide Alkaloids

Ergopeptide (ergovaline plus ergovalinine) results are reported for tall fescue alone (Figures 1 and 2) and for the composite pasture sample (Figures 3 and 4). Seasonal trends for total ergopeptides in tall fescue were low (< 0.500 ppm) prior to 11 April and peaked between 23 and 30 May (Figures 1 and 2). The trend was similar in fields

Figure 1. Seasonal levels of ergopeptide alkaloids in tall fescue alone from fields without BCT.

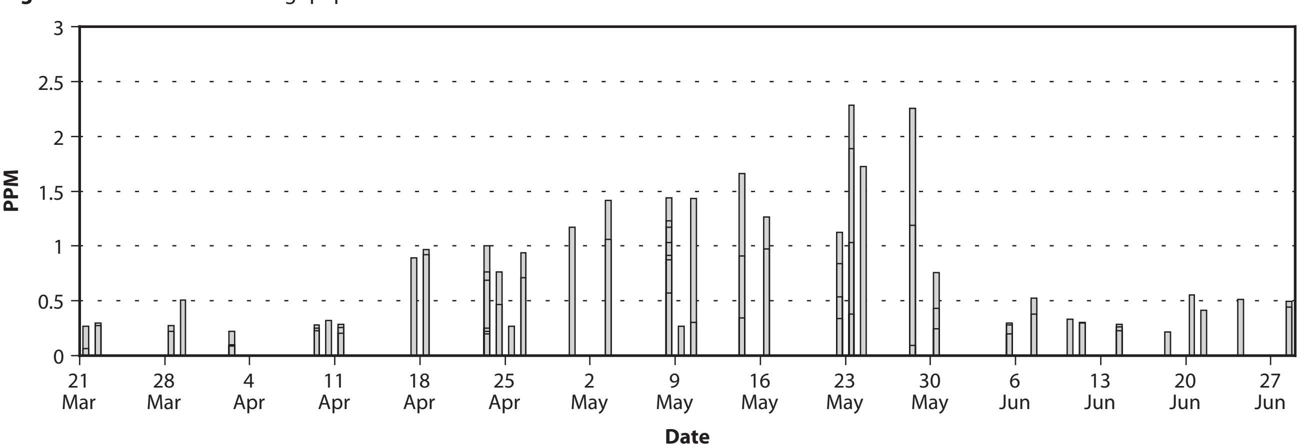


Figure 2. Seasonal levels of ergopeptide alkaloids in tall fescue alone in fields with BCT.

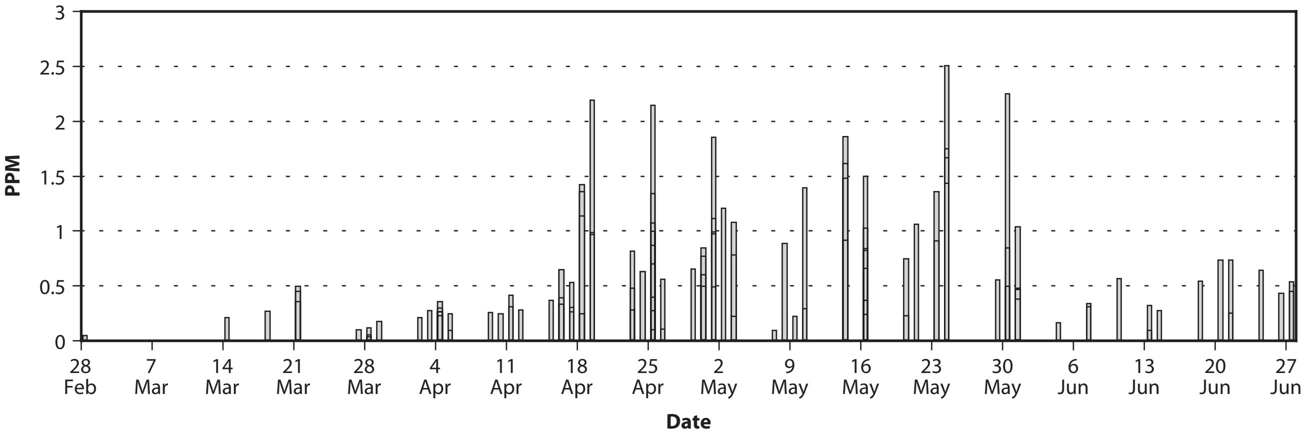


Figure 3. Seasonal concentrations of ergopeptide alkaloid concentration in total pasture forage in fields without BCT.

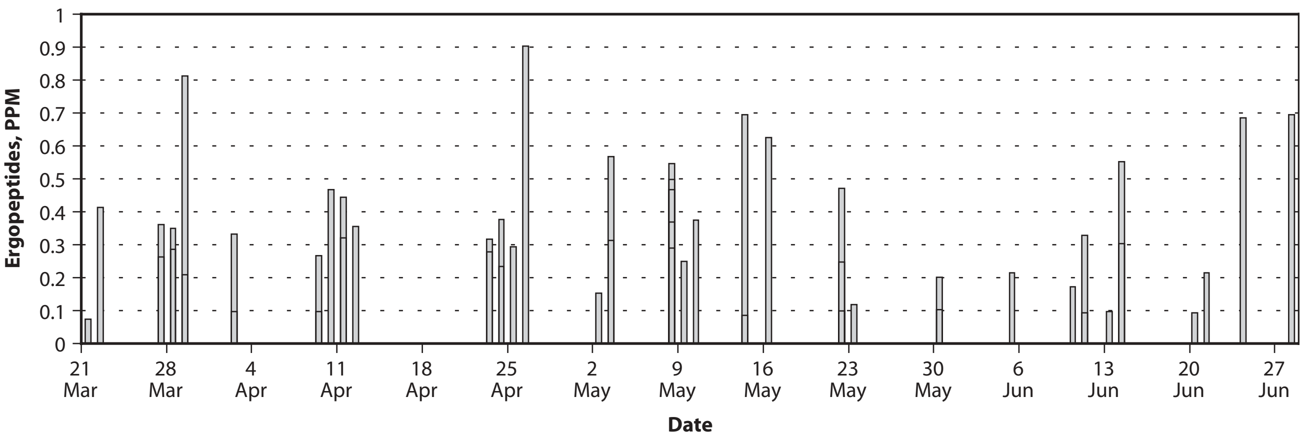


Figure 4. Seasonal concentrations of ergopeptide alkaloid concentration in total pasture forage in fields with BCT. Values are averages of samples in and out of proximity to BCT.

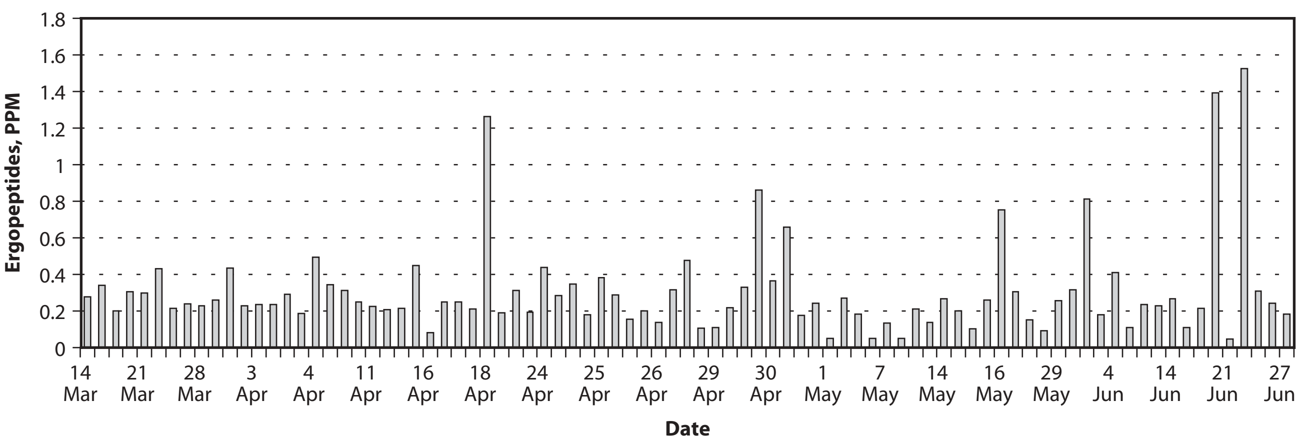


Figure 5. The effect of ergopeptide alkaloid content of tall fescue alone on EFL and LFL across all fields.

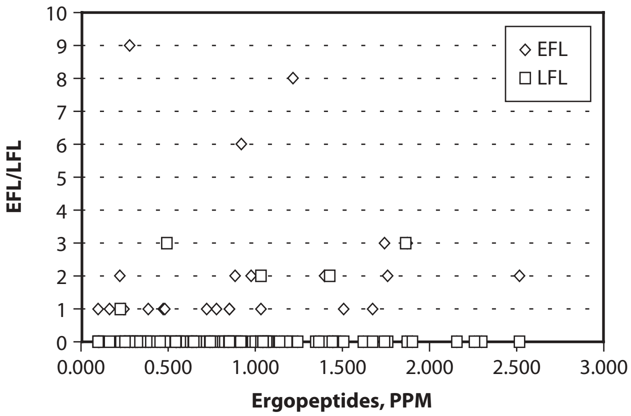
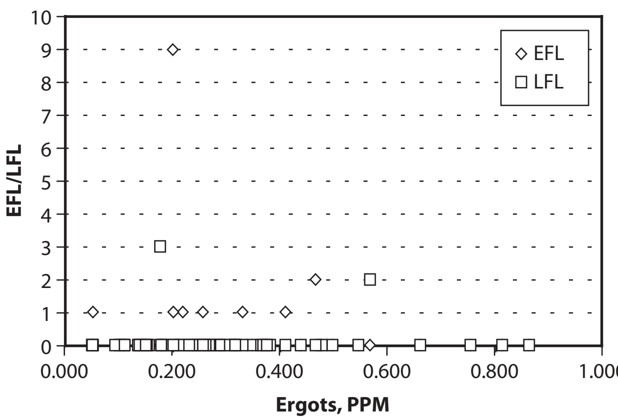


Figure 6. Effect of ergopeptide alkaloid concentration in total pasture forage on EFL and LFL across all fields.



with and without BCT. In two instances in late June, the total ergot alkaloid levels were higher in the composite pasture samples than in the tall fescue alone (Figure 4 versus Figure 2). EFL and LFL did not increase with rising ergopeptide content in either tall fescue alone or the total pasture (Figures 5 and 6).

Fungal Mycotoxins

Only 63 of 402 samples had measurable dioxynivalenol (DON), T2, or zearalenone (ZEA) levels, and fields without BCT had positive mycotoxin results more frequently than those with (Tables 1 and 2). The majority of MRLS losses occurred on fields with no detectable mycotoxins (Table 3).

Soil Microbiology

Total yeasts and molds (Y+M) were variable and did not exhibit a seasonal trend with total populations between 10^5 and 10^6 colony-forming units per gram (CFU g^{-1}) (Figures 7 and 8). Counts tended to be greater in fields without BCT. Total Y+M counts did not impact MRLS incidence (Figure 9).

Nitrate, Forage Minerals, and Cyanide

Levels of nitrate-nitrogen (NO_3-N) were generally less than 1,000 ppm and 1,500 ppm for fields with and without BCT, respectively (Figures 10 and 11). Fields with BCT had more occurrences greater than 1,000 ppm than those without. While significant EFL losses did occur in some fields with high nitrate levels, nitrate had no consistent relationship with MRLS symptoms (Figure 12).

Forage samples from all pastures were analyzed for calcium (Ca) and potassium (K) content. In addition, routine soil tests were made for each field as it became part of the monitoring program. Limited numbers of samples were analyzed for sodium (Na), but these were uniformly low (approximately 40 ppm, data not shown). One theory of MRLS in 2001 was a mineral imbalance between K and Ca in forage, possibly exaggerated by late freeze events. Season-long values for potassium:calcium (K:Ca) ratio varied between

Figure 7. Seasonal populations of yeasts and molds in fields without BCT.

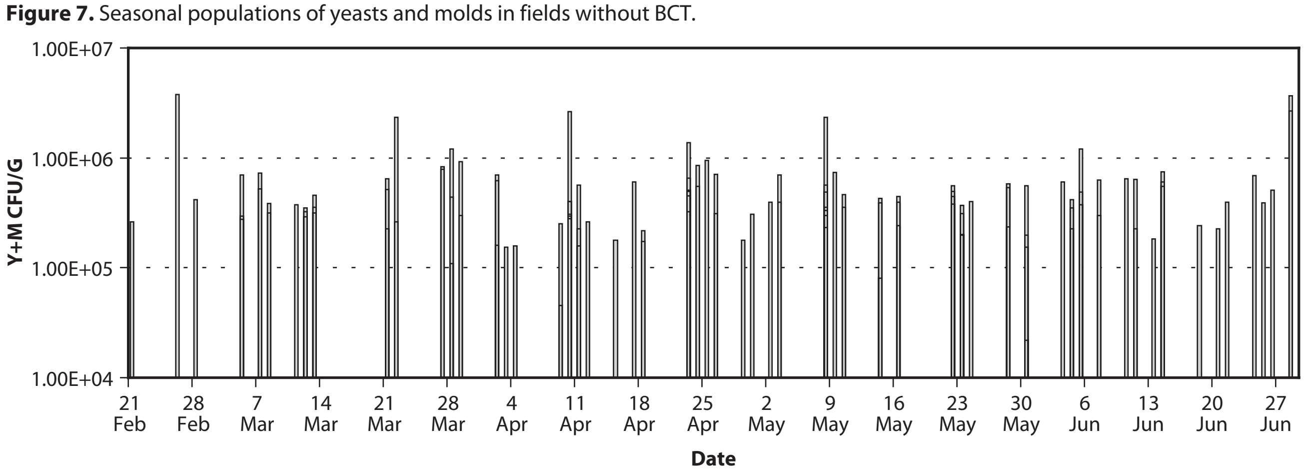


Table 1. Seasonal mean, minimum and maximum levels of mycotoxins present in fields with BCT (ppb). Values are averages of samples in and out of proximity to BCT.

	DON			T2			ZEA		
Week of	**mean**	**min**	**max**	**mean**	**min**	**max**	**mean**	**min**	**max**
18-Feb									
4-Mar									
18-Mar									
1-Apr									
15-Apr				131	100	173	469	356	574
29-Apr	733	700	1100	173	104	205	420	420	420
13-May	850	800	900	122	122	122			
27-May	833	700	1100	157	104	189			
10-Jun	600	600	600						
24-Jun	600	600	600	138	138	138			

Table 2. Seasonal mean, minimum and maximum levels of mycotoxins present in fields without BCT (ppb).

	DON			T2			ZEA		
Week of	**mean**	**min**	**max**	**mean**	**min**	**max**	**mean**	**min**	**max**
18-Feb									
4-Mar									
18-Mar									
1-Apr									
15-Apr				143	123	171	449	349	548
29-Apr				148	112	202			
13-May				126	103	149			
27-May	850	800	900	166	112	220			
10-Jun	700	700	700						
24-Jun				132	132	132			

Figure 8. Seasonal populations of yeasts and molds in fields with BCT. Values are averages of samples in and out of proximity to BCT.

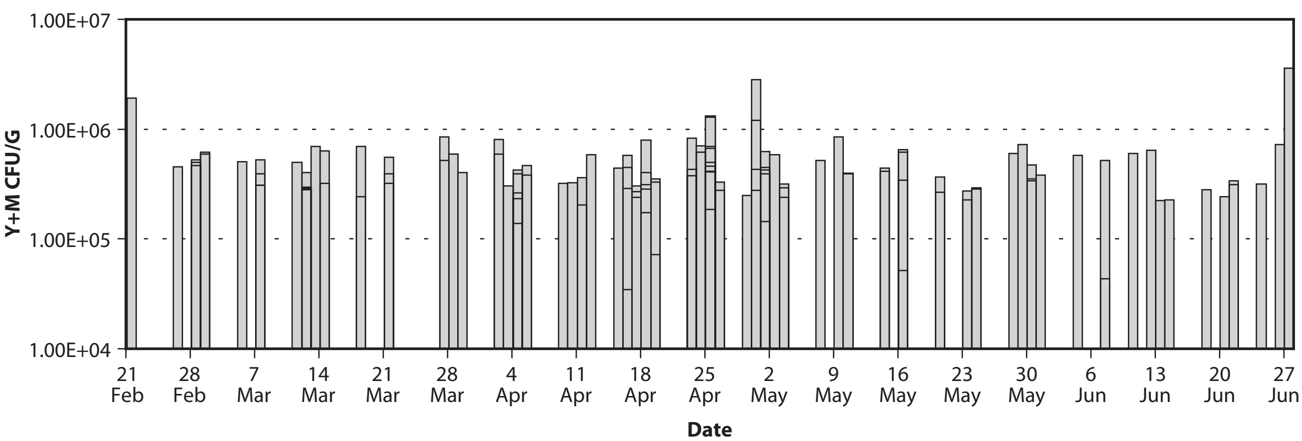


Figure 9. Effect of yeast and mold count on EFL and LFL across all fields.

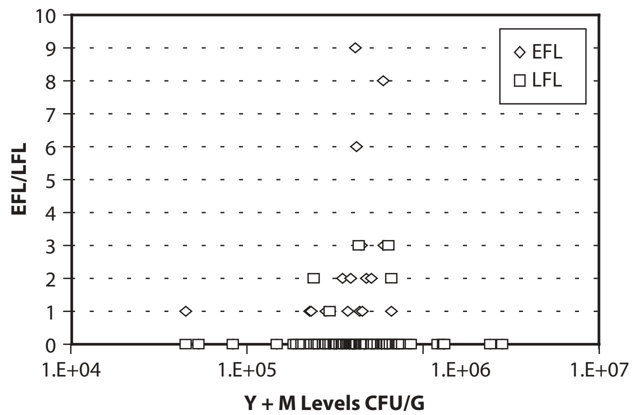


Figure 10. Seasonal nitrate nitrogen concentrations in total pasture forage in fields without BCT.

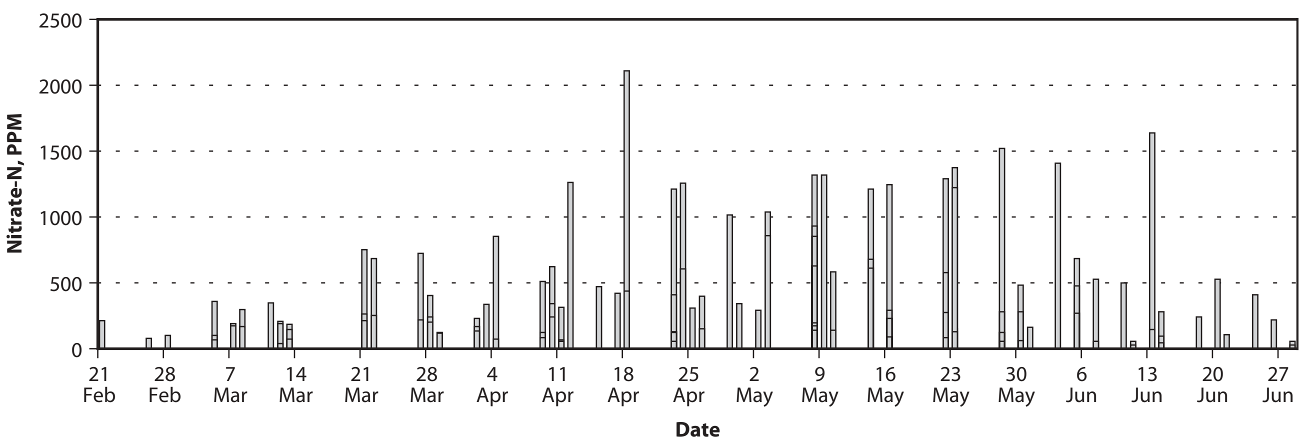


Figure 11. Seasonal nitrate nitrogen concentrations in total pasture forage in fields with BCT. Values are averages of samples in and out of proximity to BCT.

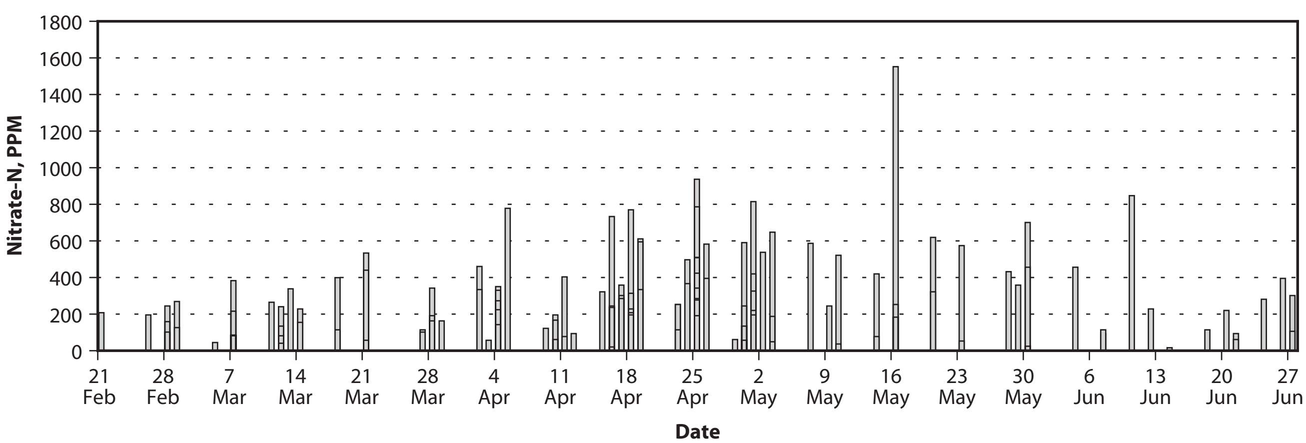


Figure 12. Effect of nitrate nitrogen on EFL and LFL across all fields.

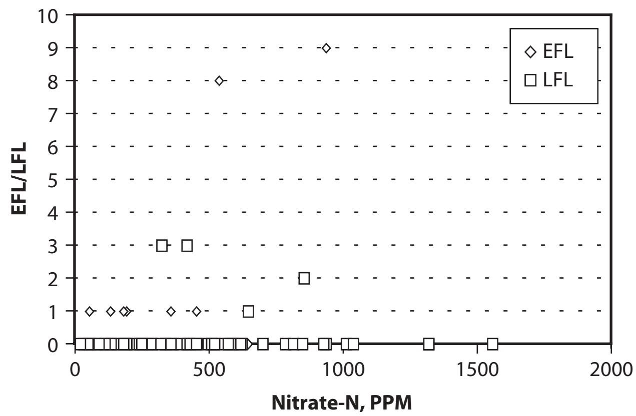


Figure 13. Seasonal K:Ca ratios for fields without BCT.

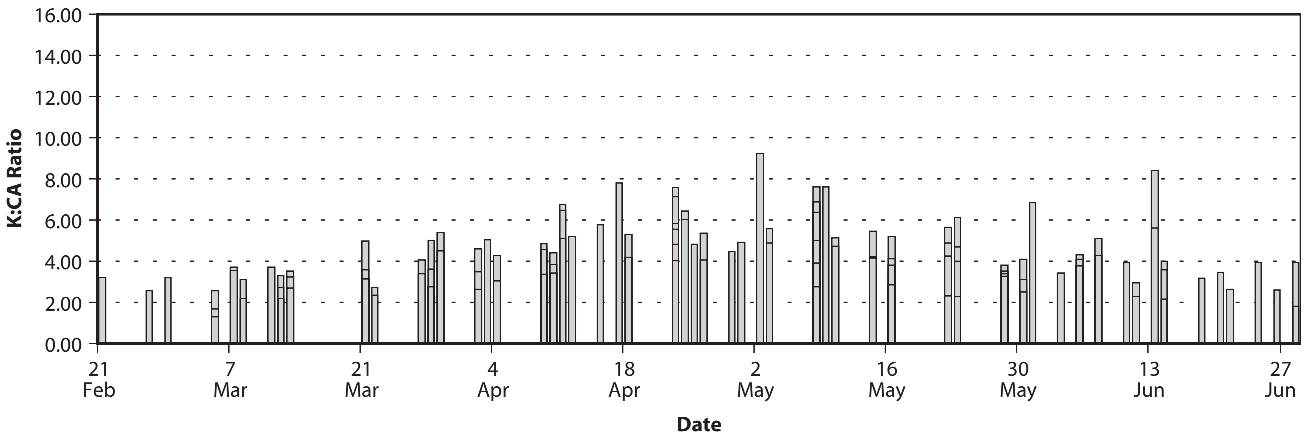


Figure 14. Seasonal K:Ca ratios for fields with BCT. Values are averages of samples taken in and out of proximity to BCT.

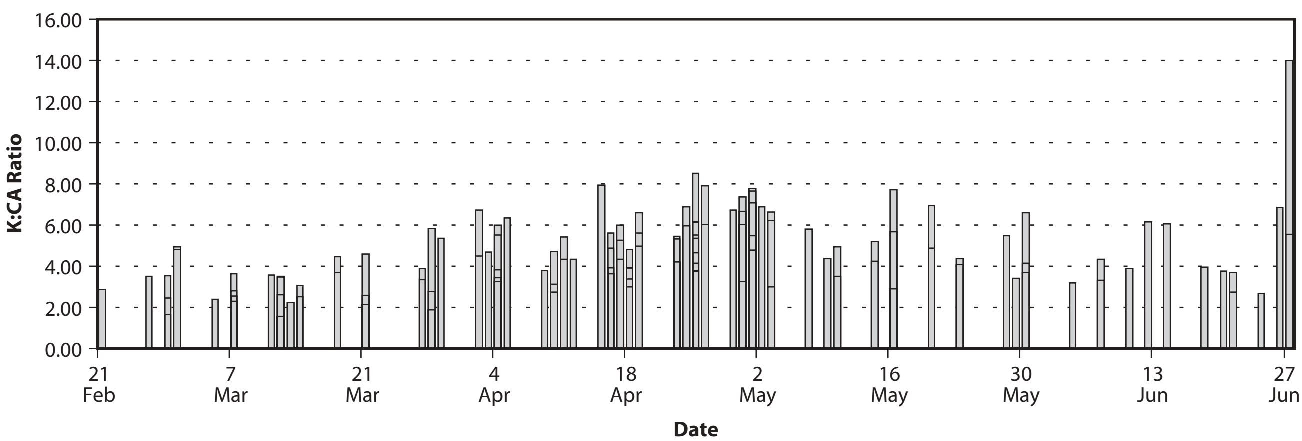


Figure 15. Effect of K:Ca ratio on EFL and LFL across all farms.

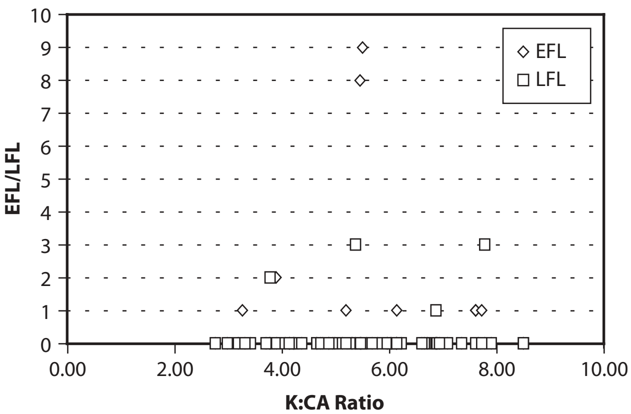


Table 3. Effect of mycotoxin level on total MRLS losses in 2002.

Mycotoxin Level	All Losses (EFL + LFL)
Below detectable limits	48
Positive DON, T2 or Zearalenone	13

Table 4. Average, minimum and maximum soil pH, Ca, K, P, Mg and Zn levels for 75 fields of the 2002 MRLS monitoring program.

	Soil pH	Buf pH	Ca, lb/A*	P, lb/A	K, lb/A	Mg, lb/A	Zn, lb/A
Avg	6.5	6.7	4861.8	309.5	665.5	407.0	7.3
Min	5.7	6.3	3092.0	58.0	252.0	197.0	2.5
Max	7.1	7.1	13076.0	740.0	1579.0	657.0	28.2

* lb/A = pounds per acre

1.31 and 14.0 and were not correlated to MRLS incidence (Figures 13, 14, and 15). Soil test levels for most fields were high to very high in Ca, K, and phosphorus (P), normal for magnesium (Mg) and zinc (Zn), and had pH values that would support good forage growth (Table 4).

Where detected, cyanide levels in white clover varied from 6.40 to 816 ppm hydrogen cyanide (HCN) and did not seem to differ in fields with or without BCT (Figures 16 and 17). Incidence of MRLS came from fields with low values for HCN (Figure 18).

Discussion

The alkaloids of tall fescue (lolines and ergopeptides) are produced in association with the presence of the endophytic fungus (*Neotyphodium coenophialum*) (1). Ingestion of tall fescue infected with the endophyte can result in foaling problems, specifically agalactia, dystocia, and prolonged gestation (2). While thresholds are unclear, levels of total ergopeptides greater than 500 to 600 ppm in the total diet are considered to induce fescue toxicosis in late gestation mares (Lowell Bush, personal communication). Several fields had tall fescue that exceeded 500 ppm ergopeptides from late April to late May (Figures 1 and 2). The assay for ergopeptide alkaloids in the composite pasture sample (taken from several points in the field without respect for species) should be more representative of the total diet of the grazing horse (Figures 3 and 4). Several composite samples exceeded 500 ppm across the season. However, no symptoms of fescue toxicosis were observed in mares grazing these pastures.

The relationship between ergopeptide alkaloids and MRLS losses is not clear, and high levels of alkaloids did not necessarily result in abortions. However, in three fields that did not have BCT but that did experience losses (5 EFL, 2 LFL), ergopeptide levels were very high (0.8 to 1.7 ppm, data not shown). Although the data are not conclusive, it appears that high ergopeptide levels may have played a role in fetal losses in 2002.

The mycotoxin theory of 2001 involved the presence of fungi in problematic pastures whose growth was stressed first by drought and then by frost leading to the produc-

Figure 16. Seasonal HCN concentrations in white clover from fields without BCT.

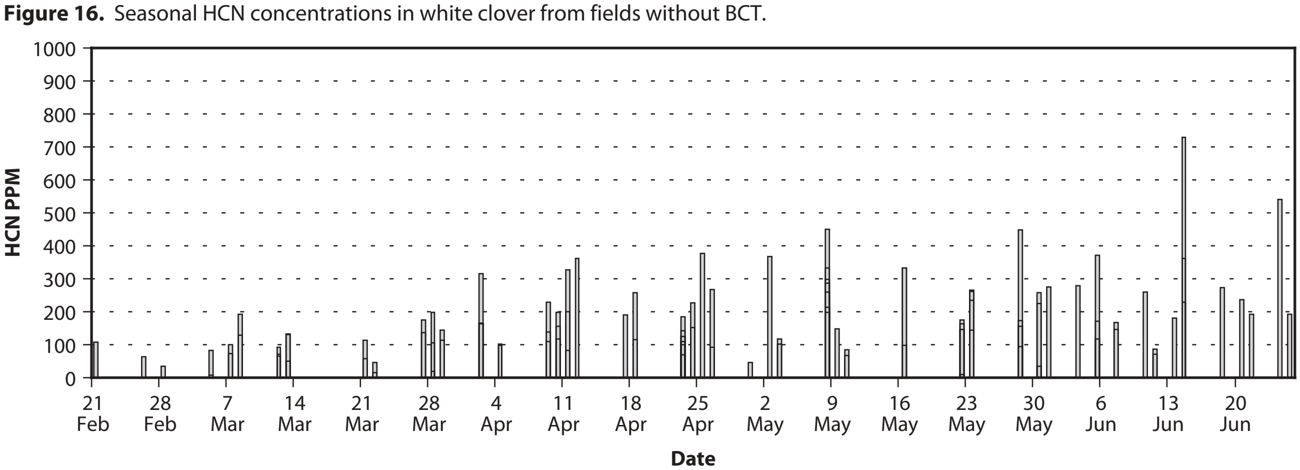


Figure 17. Seasonal HCN concentrations in white clover from fields with BCT. Values are averages of white clover samples taken in and out of proximity to BCT.

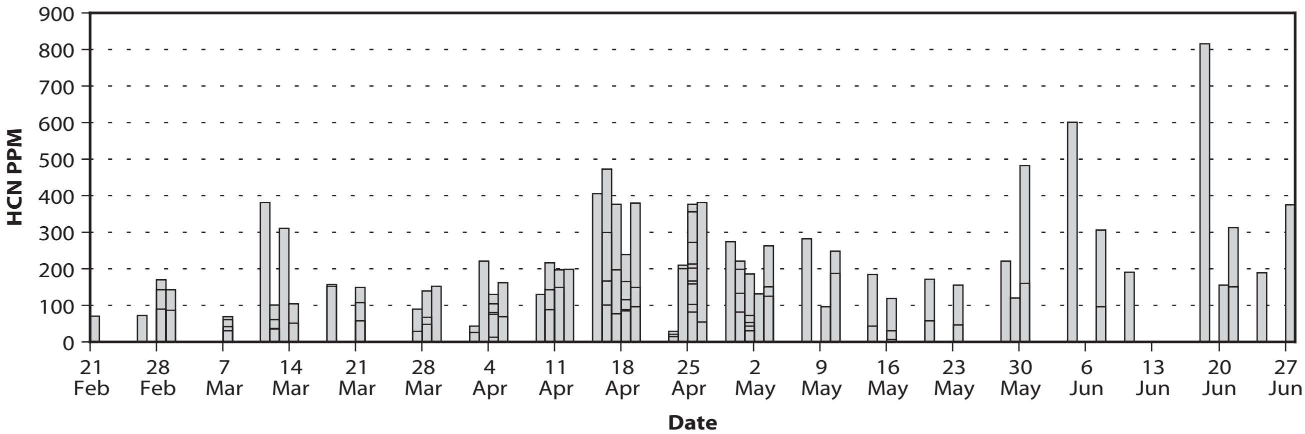
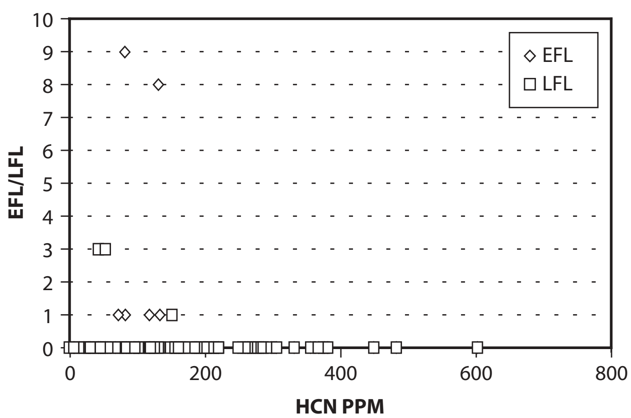


Figure 18. Effect of HCN concentration in white clover on EFL and LFL across all fields.



tion of mycotoxins that could cause MRLS. In 2001, rain events shortly after the Kentucky Derby hampered the ability to collect samples that would have been representative of the diet of mares experiencing MRLS (mycotoxins are water soluble). Therefore, it remained unclear if mycotoxins were produced in 2001 and what role they might have played in MRLS. Mycotoxins produced by *Fusarium* spp. were suspected but never found from the limited number of samples taken in 2001 during the MRLS episode. Samples taken in 2002 found that very few samples tested positive for mycotoxins, and these were not correlated with MRLS symptoms (Table 3).

Monitoring yeast and mold populations of the soil of horse pastures was thought to potentially predict problems should the conditions occur that favor mycotoxin production. Although fields with fetal losses were on the upper end of Y+M populations, there was not a clear relationship between fungal counts and MRLS occurrence (Figure 9). Therefore, it is doubtful that following microbial populations will provide a means of predicting the occurrence of MRLS.

Nitrate-nitrogen values of forage from monitored pastures increased from early March to some time in mid-April where the values seemed to plateau (Figures 10 and 11). In general, the values for nitrate content are higher than those reported during May of 2001 (Roger Allman, personal communication). The maximum amount of nitrate that horses can tolerate in their diet is not known, but Lewis reports that 4,090 to 4,770 ppm NO_3-N is known to be safe for pregnant or lactating mares (3). The values found in monitored pastures were well below this threshold (Figures 10 and 11). Finally, NO_3-N was not a predictor of EFL or LFL in 2002 (Figure 12).

The mineral hypothesis for MRLS advanced in 2001 was that a diet high in potassium, proteins, and carbohydrates and low in calcium and sodium chloride induced a mineral and electrolyte imbalance resulting in immune suppression and reproductive loss in mares. The suppression of the immune system would then lead to the overgrowth of the microorganisms found to be consistent with many cases of MRLS. Specifically, the K:Ca ratio was pointed to as the indicator of a problematic situation. In addition, it was hypothesized that the drought of 2001 and the late frosts would stress the plant leading to greater mobilization of potassium and hence greater K:Ca ratios. The K:Ca ratio ranged widely on monitor farms from 1.56 to 14 (Figures 13 and 14). These values were similar to those reported in May of 2001 (Roger Allman, personal communication). However, K:Ca ratio was not a good predictor of MRLS incidence (Figure 15).

The release of HCN from white clover (*Trifolium repens* L.) depends on the occurrence of cyanogenic glycosides (linamarin and/or lotaustralin) and the enzyme beta-glucosidase (4). Ordinarily these are compartmentalized, and no HCN is released. However, when the tissues are damaged (such as by frost), the substrate(s) and enzyme mix, releasing HCN. The level of cyanogenic glycoside in white clover is genetically determined and varies by season. Many MRLS pastures in 2001 were characterized by higher than normal proportions of white clover. It was hypothesized that the late frost events that were coincidental with the onset of MRLS symptoms might have been related to release of HCN from white clover leaves damaged by the low temperatures. Cyanide release by white clover is increased by drought and by low temperatures (5). Mares were also observed to graze the clover areas preferentially in problematic pastures.

In 2002, cyanide levels in white clover ranged from 0.1 to 816 ppm across all fields (Figures 16 and 17). Crush and Caradus reported a range of 120 to 1,110 ppm HCN in 51 white clover cultivars grown under greenhouse conditions (4). In general, the cultivars of white clover used in the United States are low in cyanide potential, but some 2002 fields had HCN levels in white clover approaching those found in New Zealand, where cyanogenic potential is desired for improved agronomic performance and pest resistance (4,6). However, the EFL/LFL losses experienced by monitoring farms in 2002 were on fields with relatively low HCN levels in the clover (Figure 18).

General Farm Observations

Routine observations were made on monitored farms or on referral farms as to what and where the mares were grazing. Any poisonous plant found was brought to the attention of farm personnel. No poisonous plants were observed being eaten or bitten. From early March until the end of June, white clover was the preferred forage for all mares. It was also noted that mares would not graze white clover if it was mixed with tall fescue. Additionally, white clover mixed with timothy, orchardgrass, or bluegrass was selected over tall fescue or tall fescue-white clover mix.

Grazing generally was observed where the white clover population was the greatest, which, in most cases, was away from fences and tree lines. During the period of ETC migration, mares were observed, in some cases, several hundred feet away from BCT while grazing. However, ETC were also observed a similar distance from their host. ETC pupae were found in great numbers on fences between the board and the post. They were also noted in orchardgrass crowns well within the grazing height of mares and cutting height of hay production equipment. This condition may explain EFL losses that occur well after the migration was completed.

Ten of the 12 monitored horse farms had aggressive management approaches for controlling/eradicating ETC. They included complete removal of BCT, removing egg masses, spraying or injecting insecticides (organic and inorganic), and burning ETC tents. Other approaches included dry lots for mares, muzzling of mares, or completely moving the mares away from BCT. Losses occurred on six of the 12 horses farms regardless of management approach. Four farms experienced losses in spite of an aggressive management plan partly because of BCT outside of their control and greater than anticipated ETC movement.

Conclusion

The excellent cooperation among farm managers, veterinarians, private consultants, and laboratories and university personnel enabled unprecedented data collection on the environmental and forage characteristics of the pasture of horse farms in Central Kentucky in 2002. Baselines were established for several parameters in a coordinated way to allow comparison for future years and for future problems. One of the major problems in dealing with the MRLS outbreak in 2001 was the inability to define what "normal" would be for a range of pasture parameters, including alkaloids in tall fescue, fungal mycotoxins, soil microbial populations, cyanide, and nitrate. These and other parameters considered as possible causal agents of MRLS were measured on 12 Central Kentucky horse farms and one hay production farm. No measured parameter was observed to have a clear relationship to MRLS in 2002. Farms and fields with losses compared to those with no losses support ETC involvement in MRLS. ETC presence was therefore a good predictor of MRLS potential. However, there was some indication of problems related to LFL from very high levels of ergopeptides in tall fescue. Finally, the data collected during 2002 indicate that the number of pasture parameters that should be followed in the future can be reduced. Still needed, however, is knowledge of the toxic principle and how it is transferred to the grazing mare. Ideally, this compound or organism could be characterized so that horse farm managers are able to make sound decisions as to the safety of pastures for pregnant mares.

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Summary

J. Henning

IT IS VERY EASY TO SUMMARIZE A SESSION WITH ONLY ONE paper, so I will just let Wayne Long's comments stand. I would like to recognize the oversight committee for their helpful advice and consultation over the past year. I would especially like to recognize the invaluable efforts of Dr. Brown and Dr. Riddle, who helped us to craft the monitoring plan and to change it in midstream.

In addition, farm managers were with us: Fred Seitz, Dan Rosenberg, Art Zubrod, as well as Rusty Ford from the Department of Agriculture. Dr. Powell was ever present with words of wisdom and was a calming force in some tense meetings.

Let me just add that I was very pleased we were able to finally get some samples ahead of an event. I was worried this year that if we did in fact control all the caterpillars that we might not have a chance to correlate field experience against the MRLS problem, not that I wanted to see one, necessarily. But we do have prior forage, blood, and urine samples, as well as samples taken from within the time when mares were losing foals, so we can go back to try to construct what happened. This situation is a great improvement over 2001 when we took samples after the fact and tried to guess what the horses did or what they were doing when they lost the foal.

We were also able to respond, through our veterinarians, to a great number of farms that were experiencing losses for reasons they could not figure out. When we did the follow-up visits, of course we were looking for the standard items. We would take samples, and some of those are included in the measures Wayne Long presented. But we would have to agree with so many of the thoughts expressed that in some cases we've got losses not proportional to numbers of caterpillars. The three fields that had less obvious cherry trees had exceptionally high ergovaline and ergovalinine numbers. That's just one of the things that always bothers you when you put a theory together and try to find out if the data fit. There are times when you've got small trees or long distances from trees to horses on farms that had problems. There are also losses on farms that were adamant that thought they were doing a good job. So we've got some apparent outliers that, when we finally understand the vectors and mechanism, will make sense.

Finally, let's talk about 2003. I think any good monitoring program ought to do three things. First, it ought to predict a problem. In 2002, it was clear because of lag of analysis time and our "shotgun" approach that we probably weren't going to predict but respond. I think with the help of these two days, we've got a lot more ideas about the parameters we can monitor. Second, it ought to provide data for any theories as to the exact cause, and I think the 2002 samples should help us. Third, it ought to help farm managers answer the question "Is my pasture safe?" There's still a big challenge out there as far as answering that question, and I'm glad for some leads. Dr. T. Fitzgerald mentioned the Serratia. I'm glad we've got Dr. Newman on the team, who's a wizard at doing the super science stuff, as is Dr. Bush. Any monitoring program ought to predict, correlate, and then, after the fact, help people know when they can go back on pastures.

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