AVOIDING WEED SHIFTS AND RESISTANCE IN ROUNDUP READY ALFALFA

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ABSTRACT

Weeds present a continual challenge to maintaining profitable alfalfa production. The Roundup Ready production system, utilizing transgenic alfalfa, has significant potential to simplify weed management, while also improving broad-spectrum control of both annual and difficult-to-control perennial weeds. Roundup has proven to be a reliable herbicide treatment in transgenic crops and has improved weed management in the short term. However, weed species shifts and the selection for Roundup-resistant weeds have resulted from the increased use of this technology. Alfalfa is especially vulnerable to these problems for several reasons: tillage in an existing crop is not practical, alfalfa is produced in large fields with a great diversity of weeds, and there is significant potential for long-term repeated use of a single herbicide because it is a perennial crop. Alfalfa growers can learn from the experience gained with other Roundup Ready crops to minimize weed shifts and the evolution of resistant weeds. Weed control systems that integrate tillage between plantings, crop rotations, rotations with herbicides of different modes of action (preferably soil residual herbicides), and tank mixtures are important. The long-term effectiveness and sustainability of the Roundup Ready system in alfalfa depends upon how well growers adopt the concept of rotation into their production systems. A preemptive approach is warranted—in other words, one should not wait until weed shifts and resistance occur before utilizing these strategies.

Key Words: *Medicago sativa*, herbicide resistance, weed species shifts, weed control, resistance management, pest management

IMPORTANCE OF WEED CONTROL

Alfalfa, the queen of forages, is an important crop throughout the Western US. It is practically an indispensable component of the feed ration for dairy cows and is a principle feed for horses, beef cattle, sheep, and other livestock. Because nearly all weeds reduce the palatability and nutritional value of alfalfa, these livestock industries—especially the dairy and horse industry—expect nearly weed-free hay. This can be difficult to achieve in many alfalfa fields when using conventional herbicides. Typically, no single herbicide controls all weeds present in a field and some weeds, especially perennials, are not adequately controlled with any of the current conventional herbicides. In addition to being difficult to achieve, complete weed control in alfalfa is costly. Growers continually seek ways to enhance the level of weed control while minimizing costs.

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DEVELOPMENT OF ROUNDUP READY ALFALFA

Glyphosate (Roundup) is generally considered the most effective broad-spectrum post-emergence herbicide available. Roundup-resistant or Roundup Ready alfalfa (RR alfalfa), developed in late 1997 and made commercially available in the fall of 2005, makes broadcast applications of Roundup to alfalfa possible. This technology imparts genetic resistance to Roundup by inserting a single gene from a soil bacterium into alfalfa. The biotechnology-derived plants have an altered enzyme that allows them to tolerate a Roundup application while susceptible weeds are killed.

This technology is a major development, providing growers with a useful weed management tool and a means to deal with some of the most difficult-to-control weed species. Beginning in 2001, trials conducted by UC Farm Advisors throughout California demonstrated the effectiveness of this technology. Roundup was especially effective for seeding alfalfa, less alfalfa injury and superior weed control when compared with other herbicides. One of the greatest advantages of this technology is that it provides a tool for suppressing perennial weeds such as dandelion, nutsedge, bermudagrass, and quackgrass that have not been adequately controlled with conventional practices.

WEED SHIFTS AND RESISTANCE

Initial experience in commercial fields has confirmed these research findings and has further demonstrated the benefits of this technology—increased ease of use and superior weed control. However, no new technology is a panacea and, like other innovations, it has its limitations. The future value of this technology may be jeopardized if it is not used wisely. The greatest area of concern with this new weed-management system is the potential for weed shifts and weed resistance.

A weed species shift is far more common than resistance. If Roundup does not control all the weeds, the tendency is to quickly jump to the conclusion that resistance has occurred. However, a weed shift is a far more likely explanation for weed escapes following an application of Roundup. (See Table 1 for a list of Roundup-tolerant weeds sometimes found in alfalfa fields). It is important to understand the distinction between a *weed shift* and *weed resistance* because the significance, as well as the management approach for dealing with each, is different.

> A **Weed Shift** refers to a change in the relative abundance or type of weeds as a result of a management practice. The management practice could be herbicide use or any other practice that brings about a change in weed species composition. In the case of chemical weed control, no single herbicide controls all weeds, as weeds differ in their susceptibility to an herbicide. Susceptible weeds are largely eliminated over time with continued use of the same herbicide leaving tolerant weeds, which often thrive and proliferate with the reduced competition. So over time, there is gradual shift or increase in tolerant weed species. A weed shift does not necessarily have to be a shift to a different species. For example, with a foliar herbicide like Roundup, there could also be a shift within a weed species to a late-emerging biotype that emerges after application.

In contrast to a weed shift, **Weed Resistance** is the inherited ability of a weed to survive an herbicide dose that kills the wild type of that species. In other words, resistance occurs when there is a genetic change so that a weed that is normally controlled by an herbicide is no longer controlled.

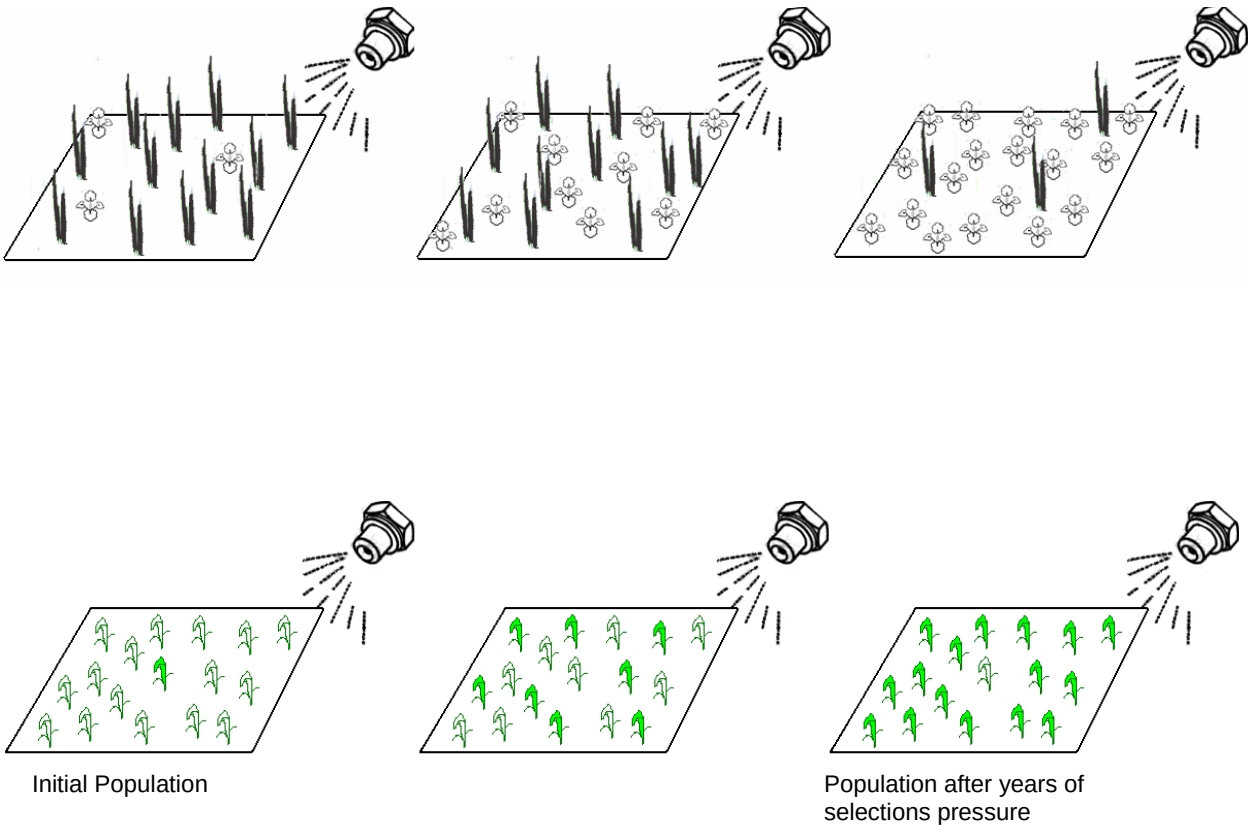


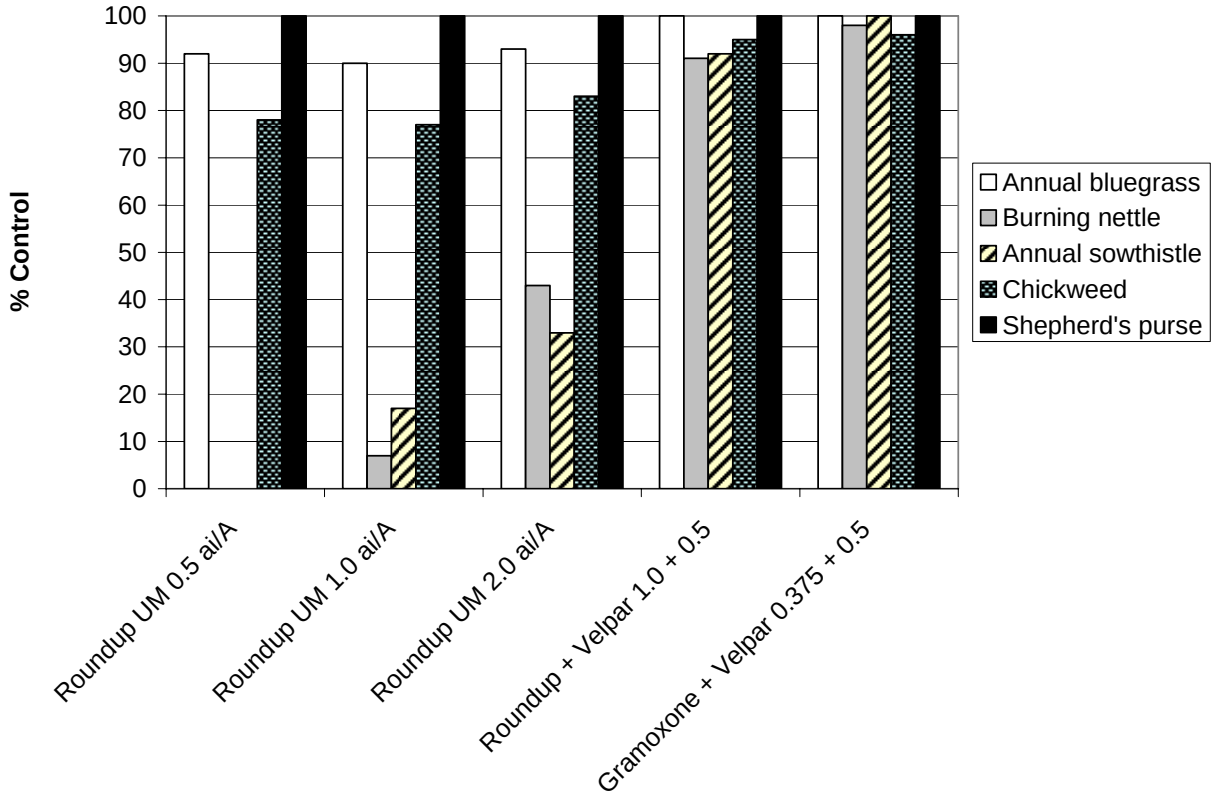
Figure 2. Herbicide resistance is a consequence of selection pressure. An herbicide controls susceptible weeds preventing them from reproducing, leaving only those individual weeds carrying the genes for resistance. Typically an extremely small percentage of the weed population initially possesses the genes for resistance. These genes may already exist in a weed population, or they may arise through mutation. As repeated use of an herbicide controls the susceptible individuals, the resistant weeds continue to multiply and ultimately become predominant.

The occurrence of weed shifts and weed resistance is not unique to genetically-engineered crops. A common misconception is that weed resistance is more likely to occur in a genetically-engineered crop field—believing that herbicide resistance is transferred from the crop to weed species. However, weed resistance is not transferred across species, so if the weed species is different from the crop species, the herbicide resistance trait cannot be passed to the weed species.

Transgenic herbicide-tolerant crops do, nonetheless, have greater potential to foster weed shifts and resistant weeds since a grower is more likely to use a single herbicide repeatedly. Continued use of the same management practice, or in this case herbicide, increases the probability of a weed shift or development of resistant weeds as a result of constant selection pressure. For example, if the herbicide diuron (Karmex) is used alone for several years in established alfalfa,

susceptible weeds are controlled, but there is likely to be an increase in tolerant weeds such as common groundsel, Persian speedwell, and others. Or, if imazethapyr (Pursuit) is used repeatedly for several years without rotating herbicides, there is likely to be an increase in prickly lettuce and many grassy weeds. The following example (Figure 3) demonstrates weed control in an established field of RR alfalfa. Annual bluegrass and shepherd's purse were adequately controlled with Roundup, whereas chickweed control was about 80 percent and burning nettle and annual sowthistle were not adequately controlled with any of the Roundup rates. In this situation, if Roundup is used repeatedly, there will be a weed species shift away from annual bluegrass and shepherd's purse to burning nettle and annual sowthistle. A tank mix of Roundup and Velpar, or a rotation to Velpar and Gramoxone, was needed to adequately control all weed species.

Figure 3. Weed control 69 days after treatment in an established stand of Roundup Ready alfalfa. San Joaquin County. 2004.



WEED SHIFTS AND RESISTANCE WITH RR ALFALFA

The possibility of weed shifts and weed resistance is particularly a concern with RR alfalfa, the first perennial crop with this transgenic trait, due to a long alfalfa stand life and the potential for repeated use of a single herbicide over several years. It is common in many areas of the northwestern US to leave an alfalfa stand in production for 5-7 years or even longer. For some growers alfalfa is the primary crop, and they want to minimize the amount of time the field is planted to low-profit rotation crops. Therefore, there may be a continuous alfalfa crop in a field

for 14 or more years with only one year of a small grain rotation crop between alfalfa plantings. Especially if the grain is not treated with an herbicide, that makes a long time with a single herbicide. Stand life is shorter in warmer production areas like the Central Valley of California (3-4 year stand life is typical), and there are more rotation crops. However, cotton and corn are commonly rotated with alfalfa, and if transgenic RR varieties are produced, this again could result in a prolonged time period where a single herbicide is used repeatedly.

There are aspects of the alfalfa production system that both favor and discourage the development of weed shifts and resistant weeds. First, rotation opportunities with a perennial crop like alfalfa are obviously significantly reduced compared with annual cropping systems. Resistance and weed shifts are believed to evolve more rapidly in crops like alfalfa that are solid seeded, lower value compared with high-value vegetables, and grown on large acreages. Mechanical weed control is generally not viable, as cultivation is not practical in a solid-seeded crop like alfalfa. Hand weeding is not considered economical. Alfalfa fields are typically large acreage, and therefore the overall weed population is often higher, and the number of individual weeds that may contain the resistant trait is greater. On the other hand, many weeds do not flourish in an alfalfa field. Alfalfa is an aggressive competitor with most weeds, and many weed species do not tolerate frequent cutting. The interval between cutting alfalfa crops is short enough that seed production for some weeds is reduced.

Weed shifts or resistant weeds are unavoidable and will eventually occur with any herbicide with repeated use. Fortunately, resistance to Roundup is not as common as resistance to many other herbicides, such as resistance to acetolactate synthase (ALS) and ACCase herbicides that have single site enzyme mechanisms of action. Why Roundup is less prone to resistance problems is not well understood, but some speculate that it could be due to the number or frequency of mutations that may be required to confer resistance to Roundup. Regardless of the mechanism for resistance to Roundup, it is not as common as resistance to other herbicides. However, cases of weed resistance to Roundup have been documented and are increasing all the time. Table 2 lists the documented cases of Roundup resistance and the year when they were first documented.

Two weed species have documented resistance to Roundup in California—annual ryegrass and marestail. Roundup-resistant ryegrass was first found in California orchards where there was a long history of continual Roundup use. Roundup resistant marestail originated in the southern San Joaquin Valley of California in orchards, vineyards, and ditch banks where tillage was no longer used and Roundup was used continuously for several years, oftentimes with multiple applications per season.

Roundup, the most effective broad-spectrum post-emergence herbicide available, is also the most widely-used herbicide in the world. It would be a shame to lose its effectiveness as a result of mismanagement. Weed shifts and/or weed resistance have occurred with the other transgenic RR crops released before RR alfalfa. This has typically occurred after approximately 6 years of continual use. Alfalfa growers can learn lessons gained from experience with these other crops as a preemptive measure to avoid, or at least minimize, problems with weed shifts and weed resistance. The obvious questions are, “What management practices can be utilized to avoid weed shifts and weed resistance, and how do we effectively utilize this new technology?” This paper will attempt to answer these questions.

WEED MANAGEMENT PROGRAM TO REDUCE WEED SHIFTS AND RESISTANCE

Roundup-resistant crops have provided growers with an easy-to-use, low-cost, and effective weed management program. However, the effectiveness of Roundup-resistant weed control systems can make growers complacent in their weed control practices. Even though this technology is highly effective, growers must follow wise weed management principles, beginning with proper weed identification and using the appropriate rate and timing for the weeds present. For example, some weeds considered somewhat tolerant to Roundup (cheeseweed, filaree, and purslane) have been effectively controlled in seedling alfalfa with Roundup, provided the proper rate was used and the application was made when the weeds were small. Research in Nebraska has demonstrated a rapid increase in lambsquarters when a low rate of Roundup (0.5 lb ai/acre) was applied, compared with a recommended rate (1.0 lb ai/acre). Just like with traditional weed management programs, the grower must be sure to use the properly-labeled rate for the weed species present and treat at the appropriate time when the weeds are still small.

Any management practice that reduces the selection pressure (in this case the selection pressure is imposed by continual use of the same herbicide) will help avoid the evolution of weed species shifts and resistance. Crop rotation is clearly one of the most effective practices. Continuous (also called "back-to-back") alfalfa is not recommended for other agronomic reasons, but especially would be ill-advised when it comes to resistance management and weed shifts. Crops differ in their ability to compete with weeds; some weeds are a problem in some crops, while other weeds are problematic in others. Crop rotation allows the usage of different weed control practices, such as cultivation and application of herbicides with different sites of action. The effectiveness of crop rotation to manage weed shifts and resistance is mostly lost if another RR crop is planted.

Weed shifts occur because herbicides are not equally effective against all weed species and differ greatly in the weed spectrum they control. A weed species that is not controlled will increase in density following repeated use of one herbicide. Therefore, rotating herbicides is recommended. Rotating herbicides reduces weed shifts, provided the rotational herbicide is highly effective against the weed species that is not controlled with the primary herbicide. The grower should rotate to an herbicide with a complimentary spectrum of weed control. Weed susceptibility charts are useful to help develop an effective herbicide rotation scheme.

Rotating herbicides is also an effective strategy for resistance management. Within a weed species there are individuals that have a different genetic makeup that enables them to survive a particular herbicide application. The susceptible weeds in a population are killed, while the resistant ones survive, set seed, and increase over time. Using an effective herbicide with a different site of action controls the resistant biotype, thus preventing reproduction and slowing the spread of the resistant biotype.

For the same reasons that rotating herbicides is effective, tank mixing herbicides is also recommended. The key is to select tank mix partners that have different target sites and that compliment each other so that when combined, they provide complete or nearly complete weed control.

RECOMMENDED WEED MANAGEMENT PROGRAM FOR RR ALFALFA

The cost of RR alfalfa seed, including the technology fee, is generally twice that of conventional alfalfa seed. Naturally, growers will want to recoup their investment as quickly as possible. Therefore, there is considerable incentive for the producer to rely solely on repeated Roundup applications alone as a weed control program. Some producers may even be inclined to shave the rates to the minimum amount that would provide acceptable weed control. While relying solely on Roundup and shaving rates may provide satisfactory results in the short term, it is a risky practice in the long run and will accelerate the evolution of weed species shifts and resistant weeds. Sound weed management practices should be employed to maintain, or at least prolong, the effectiveness of this technology.

Roundup Ready alfalfa is still a relatively new technology, so therefore there has been limited field experience to date. The following are some suggestions to consider based upon proven resistance management strategies, our understanding of alfalfa production practices, and our initial experience with RR alfalfa. Ultimately, growers and pest control advisors hold the key to avoiding weed shifts and resistance by reducing selection pressure, which is accomplished by developing a weed management program that doesn't rely on continuous use of Roundup.

Controlling weeds when alfalfa is in the seedling stage is the most challenging. This is true because alfalfa is most vulnerable to weed competition when it is in this stage, as oftentimes weeds are more vigorous and competitive. Additionally, complete weed control in seedling alfalfa is often difficult to achieve and frequently requires tank mixes of different herbicides to control the broad spectrum of weeds found in an individual field. Crop injury and yield loss are also usually far greater in seedling than in established alfalfa. Numerous field trials throughout the country have proven the effectiveness of Roundup in herbicide-resistant transgenic alfalfa. Superior weed control with no perceptible alfalfa injury has occurred in most studies. Therefore, it is only logical to use Roundup for weed control in transgenic seedling alfalfa for the cost savings, improved weed control, reduced crop injury, and to eliminate the alfalfa seedlings that do not carry the Roundup resistance gene.

Ordinarily, the 1.0 pound active ingredient rate (22 oz of Roundup UltraMax) is sufficient. However, a higher rate may be needed if the field contains some of the tolerant weeds listed in Table 1. A tank mix may be advised if especially tolerant weeds are present. For example, a tank mix with Raptor or Pursuit may be advised if burning nettle is present, or a tank mix with Prism may be necessary if the field contains ryegrass.

Alfalfa stand life varies considerably throughout the West depending on the production area, grower practice, and the existence of profitable rotation crops. However, a stand life of 3 to 4 years is common in the Central Valley of California and other warm long growing-season areas of the Southwest. A stand life of 5 to 7 years is common in much of the Northwest, and some alfalfa stands remain in production in excess of 10 years. For the principles outlined above, it is unwise to rely solely on Roundup applications for weed control throughout the life of a transgenic alfalfa field. This practice would encourage weed shifts and resistance, and over time weed control would diminish in most cases. Once an herbicide is rendered ineffective as a result

of resistant weeds, its usefulness as a weed management tool is greatly diminished. After a resistant weed population has gained a foothold, it is practically impossible to eliminate.

Most alfalfa producers treat alfalfa during the dormant season for winter annual weeds that infest the first cutting. It is strongly recommended that growers not rely solely on Roundup for their winter weed control program for the duration of the stand. They should rotate to another herbicide or tank mix at least once in the middle of the life of a stand, and perhaps twice if the stand life is over 5 years.

Fortunately, all of the herbicides currently registered in alfalfa, and there are several to choose from, have a different target site of action than Roundup. The soil-residual herbicides applied during the dormant season to established alfalfa [such as hexazinone (Velpar), diuron (Karmex) and metribuzin (Sencor)] would be appropriate herbicides for a rotation or tank-mix partner. The rotation herbicide or tank-mix partner of choice depends on the weeds present in the field and their relative susceptibility to the herbicides. Paraquat (Gramoxone) is another candidate for rotation or tank mixing, but Gramoxone, like Roundup, is only a contact herbicide and is applied late in the dormant season. By rotating Gramoxone with Roundup, growers could potentially be selecting for early-emerging weeds that may be too large to control at the typical timing for Roundup or Gramoxone. Or, they could be selecting for late emerging weeds that germinate after the application.

Weed control in the last year of an alfalfa stand is often challenging because the stand is typically less dense and competitive, and there are also fewer herbicide options to choose from due to the plant-back restrictions associated with many of the soil-residual herbicides. Therefore, Roundup is a good choice for controlling weeds in the final year of an herbicide-resistant alfalfa field. This underscores the importance of having rotated herbicides before the final year so that Roundup will remain effective and control the majority of the weeds in a last-year alfalfa stand.

Summer annual grassy weeds (such as yellow and green foxtail, barnyardgrass, cupgrass and jungle rice), and sometimes broadleaf weeds like pigweed, can be problematic in established alfalfa. These weeds emerge over an extended time period whenever soil temperatures and moisture are adequate, typically from late winter or early spring—as early as February in the Central Valley—throughout the summer. They may emerge between alfalfa cuttings, so several applications may be necessary for a foliar herbicide like Roundup to provide season-long control. Multiple applications of a single herbicide during a season is cited as promoting weed resistance. Therefore, growers should not rely solely on Roundup for summer grass control for multiple seasons. It still remains to be seen how many applications of Roundup will be required for season-long summer grass control. In long growing-season areas, as many as two to three applications per season may be needed. Rather than making multiple applications of Roundup, a better approach may be to apply the pre-emergence herbicide trifluralin (TR-10), a soil residual dinitroaniline, and follow-up with Roundup later in the season as needed for escapes. Not only is this approach more in line with management practices to avoid weed shifts and resistance, but also it may be more economical, as well, compared with multiple applications of Roundup.

The practice of rotating herbicides or applying tank mixtures should be done for both dormant applications aimed at winter annual weeds and for spring/summer applications intended to control summer annual weeds. For example, rotating to Velpar for winter annual weed control for a year does nothing to prevent weed species shifts or the development of resistance in the summer annual weed spectrum. Herbicides for summer annual weed control should be rotated as well.

There is no definitive rule on how often herbicides should be rotated. The suggestion to rotate or tank mix at least once in the middle years of the life of a stand (or more often for long-lived alfalfa stands) is only a suggestion. The key point, which cannot be overemphasized, is the importance of diligent monitoring for weed escapes. Producers should stay alert to the development of weed species shifts and resistant weeds. If the relative frequency of occurrence of a weed species increases dramatically, chances are that it is tolerant to Roundup and immediately rotating herbicides or a tank mixing is advised. If a few weeds survive among a weed species that is normally controlled easily with Roundup, it could be an indication of weed resistance, assuming misapplication and other factors can be eliminated as possible causes. In these situations, it is imperative to prevent reproduction of a potential resistant biotype. Treat weed escapes with an alternative herbicide or other effective control measure.

CONCLUSIONS

The Roundup Ready production system has potential to simplify weed management, while also improving the spectrum of weed control. However, growers should learn from the experience gained in other crops and stay alert to the development of weed shifts and resistant weeds. The key is for growers to reduce selection pressure—not to rely on repeated applications of Roundup year-after-year, application-after-application. Rotate crops, rotate herbicides and utilize tank mixes as needed, depending on the weed species and weed escapes present. A grower should not wait for there to be a problem before he employs these practices; a preemptive approach is strongly encouraged.

Table 1. Roundup-tolerant annual weeds found in alfalfa fields.

Common name	**Latin name**
Annual sowthistle	*Sonchus oleraceus*
Black mustard	*Brassica nigra*
Burning nettle	*Urtica urens*
Clover	*Trifolium spp.*
Filaree	*Erodium spp.*
Henbit	*Lamium amplexicaule*
Jubglerice	*Echinochloa colona*
Knotweed	*Polygonum arenastrum*
Lambsquarters	*Chenopodium album*
Lovegrass	*Eragrostis*
Malva (cheeseweed)	*Malva parviflora*
Ryegrass	*Lolium multiflorum*
Purslane	*Portulaca oleracea*
Wild buckwheat	*Polygonum convolvulus*
Willowherb, panicle	*Epilobium brachycarpum*

Table 2. Roundup-resistant weed populations from the International Survey of Herbicide Resistant Weeds http://www.weedscience.org/in.asp\

Resistant weed	**Common name**	**Location**	**Year**
Amaranthus palmeri	Palmer Amaranth	USA (Georgia)	2005
Amaranthus rudis	Common Waterhemp	USA (Missouri) *Multiple - 2 MOA's	2005
Ambrosia artemisiifolia	Common Ragweed	USA (Arkansas)	2004
		USA (Missouri)	2004
Conyza bonariensis	Hairy Fleabane	South Africa	2003
		Spain	2004
		Brazil	2005
Conyza canadensis	Horseweed	USA (Delaware)	2000
		USA (Kentucky)	2001
		USA (Tennessee)	2001
		USA (Indiana)	2002
		USA (Maryland)	2002
		USA (Missouri)	2002
		USA (New Jersey)	2002
		USA (Ohio)	2002
		USA (Arkansas)	2003
		USA (Mississippi)	2003
		USA (North Carolina)	2003
		USA (Ohio) *Multiple - 2 MOA's	2003
		USA (Pennsylvania)	2003
		Brazil	2005
		USA (California)	2005
Eleusine indica	Goosegrass	Malaysia *Multiple - 2 MOA's	1997
Euphorbia heterophylla	Wild Pointsettia	Brazil	2005
Lolium multiflorum	Italian Ryegrass	Chile	2001
		Chile	2002
		Brazil	2003
		USA (Oregon)	2004
Lolium rigidum	Rigid Ryegrass	Australia (Victoria)	1996
		Australia (New South Wales)	1997
		USA (California)	1998
		Australia (South Australia)	2000
		South Africa	2001
		South Africa *Multiple - 2 MOA's	2003
Plantago lanceolata	Buckhorn Plantain	South Africa	2003
Sorghum halepense	Johnsongrass	Argentina	2005

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