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## IN THIS ISSUE

- Glyphosate-Resistant Horseweed (*Conyza canadensis* L. Cronq.)  
Biotype found in the Central San Joaquin Valley.....1
- Reflective Bed Mulch but not Over-the-Canopy Shade Cloth  
Controls Weeds in Field-Grown Zinnias.....5
- Strategies and Tactics for Fumigating Clay Loam Soils.....7

**GLYPHOSATE-RESISTANT HORSEWEED (*Conyza canadensis* L. Cronq.) BIOTYPE FOUND IN THE CENTRAL SAN JOAQUIN VALLEY.** *Anil Shrestha, Statewide IPM Program, Kearney Agricultural Center; Kurt Hembree and Neil Va, UCCE Fresno County*

*Key words:* Horseweed, marestail, glyphosate, resistance.

### Abstract

Glyphosate-resistant horseweed biotypes have been reported in 10 states in the U.S., mainly in annual row-crop systems. This study showed that glyphosate-resistant (R biotype) horseweed also exists on canal banks in the central San Joaquin Valley and California is now the 11<sup>th</sup> state to report glyphosate-resistant horseweed. The level of resistance to glyphosate, however, was influenced by the stage of growth of horseweed at the time of glyphosate application. There was a probability of controlling some of the 'R' biotype horseweed at the 5-8 leaf stage with a 2x (2 lb ai/ac) or 4x (4 lb ai/ac) rate of glyphosate. After the 18-21 leaf stage, the horseweed plants were able to survive glyphosate application rates up to 4x. At later stages, even some plants of the susceptible (S) biotype escaped the lower rates of glyphosate. Therefore, it is important to control horseweed at an early stage of growth. This is the first case of a glyphosate-resistant horseweed biotype existing in a non-crop situation. Close monitoring and an integrated weed management program will have to be implemented to manage glyphosate-resistant horseweed biotypes in the central San Joaquin Valley.

## Introduction

Herbicide resistance is defined by the Weed Science Society of America as “the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type. In a plant, resistance may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis.” Herbicide resistance was first reported in 1957. Since then, 304 weed biotypes have developed resistance to several groups of herbicides including glyphosate (Heap, 2005).

Glyphosate [*N*-(phosphonomethyl)glycine] is a non-selective, broad spectrum, systemic, post-emergence herbicide. This herbicide kills weeds by metabolic disruptions in the plant (Franz et al. 1997). It inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) which is essential for biosynthesis of aromatic amino acids (Mueller et al. 2003). Because of the complex manipulations of the target EPSPS enzyme required for developing glyphosate-resistant crops, it was commonly believed that weeds developing resistance to glyphosate was improbable (Bradshaw et al. 1997). However, it has been repeatedly found that development of herbicide resistance in weed populations is greatly increased by repeated use of a single herbicide (Holt, 1992). Eventually, glyphosate resistance was documented in rigid ryegrass (*Lolium rigidum* L.) in 1996 in Australia (Powles et al. 1998). Since then, 15 weed species, including horseweed (*Conyza canadensis* L. Cronq.), are reported to have developed resistance to glyphosate (Nandula et al. 2005).

Horseweed or maretail is an annual plant belonging to the Asteraceae family and it is native to North America (Weaver, 2001). The first case of a glyphosate-resistant horseweed in North America was reported from Delaware in 2000 (VanGessel, 2001). Since then, nine other states in the U.S. have reported the occurrence of glyphosate-resistant horseweed (Heap, 2005). All of these cases were from annual row-crop systems, viz. glyphosate-resistant cotton and soybean. It is believed that intensive use of glyphosate in glyphosate-resistant crops has caused the evolution of several weed populations with natural resistance to glyphosate (Nandula et al. 2005). No case of glyphosate-resistant horseweed has been reported in California or in non-crop areas. Poor control of horseweed with glyphosate was reported on an irrigation canal bank in Dinuba, CA and glyphosate resistance suspected (personal communication J. Heringer). Glyphosate was used repeatedly at this site for the past 15 years. Therefore, the objectives of this

study were to confirm the existence of glyphosate-resistant horseweed in seeds collected from Dinuba, CA and to evaluate the interaction of glyphosate rate and plant growth stage.

## Materials and Methods

Horseweed seeds were collected from a suspected glyphosate-resistant population in Dinuba, CA and a suspected susceptible population in western Fresno, CA in the fall of 2004. Seeds were stored at room temperature in the lab. On April 4, 2005, seeds were planted in plastic germination trays in the lab and moved to a greenhouse soon after emergence (April 13, 2005). The seedlings were allowed to establish and then transplanted into plastic pots (6 inches deep, 4 inches wide) containing a potting mix (Promix 5, Sungro Horticulture, Canada) on May 5, 2005. The suspected glyphosate-resistant plants were designated as ‘R’ biotype and the suspected susceptible biotype was designated as ‘S’ biotype. One seedling of each biotype was planted in each pot for a total of 200 pots. Of these, 80 pots were assigned to each of five greenhouse benches as five replications. The 80 pots were then separated into five groups of ‘R’ and ‘S’ biotype. The group designations were based on the plant growth stage for glyphosate application as follows:

1. Glyphosate spray at 5-8 true leaf stage of horseweed.
2. Glyphosate spray at 11-15 true leaf stage of horseweed.
3. Glyphosate spray at 18-21 true leaf stage of horseweed.
4. Glyphosate spray at bolting to 6 inch (height) stage of horseweed.
5. Glyphosate spray at 6 inch to 1 foot (height) stage of horseweed.

A fully expanded leaf was considered a true leaf. Within each group, the pots were further divided into four sub-groups, each to receive 0 (no glyphosate, control), 1x (1 lb ai/ac glyphosate), 2x (2 lb ai/ac), and 4x (4 lb ai/ac). Roundup Weathermax® (contains 5.5 lb ai/gal of glyphosate) was the herbicide used in the study. Therefore, within each replication, there were 40 pots each of ‘R’ and ‘S’ biotype divided into five growth stages and four herbicide rates. Each pot containing a seedling was an experimental unit. The experimental design was a two factor (glyphosate rate and plant growth stage), completely randomized block with five replications. The plants were watered regularly and fertilized twice during the growing season with a

commercial fertilizer (Miracle Gro, 4 g per gallon of water).

Glyphosate was applied at the designated growth stage of the plants with a CO<sub>2</sub> back-pack sprayer (Figure 1). The spray was discharged through a 40" boom, with a single flat-fan nozzle (TeeJet XR8002EVS) in the center and a blank at each end, 18" above the target plants. The system was pressurized to 30 psi to deliver the herbicide solution at 35 gpa (broadcast acre basis) in a 20" band. The plants were moved outside the greenhouse, sprayed, and moved back to the greenhouse after the spray dried on the leaves. The survival of each plant was evaluated weekly and data were recorded as 'alive' or 'dead'. The plants were designated as 'dead' when the above-ground plant parts started disintegrating and showed no traces of green tissue. Data were compiled as percent 'dead' or 'alive' plants and analyzed using GLM procedures in SAS. The level of significance used for the analysis was 0.05. The experiment is being repeated.



Figure 1. Glyphosate application with a back-pack sprayer.

## Results and Discussion

The 'R' and 'S' biotypes differed significantly in their ability to survive the various glyphosate treatments. A significant interaction occurred between horseweed growth stage and glyphosate application rate for both biotypes. When glyphosate was applied at the 5-8 leaf stage of horseweed, 100% of the 'R' biotype plants

survived the 1x glyphosate rate (Figure 2a). Survival of the plants was reduced to 60% at the 2x rate, while none of the horseweed plants survived the 4x rate. None of the 'S' biotype plants survived any of the glyphosate application treatments (Figure 2a).

When glyphosate was applied at the 11-15 leaf stage of horseweed, 100% of the 'R' biotype plants survived the 1x and 2x glyphosate rates (Figure 2b) and 40% survived the 4x rate. Unlike the 5-8 leaf stage, 20% of the 'S' biotype plants survived the 1x glyphosate treatment (Figure 2b). At the 18-21 leaf stage of horseweed, 100% of the 'R' biotype plants survived the 1x and 2x glyphosate rates and 80% of plants survived the 4x rate (Figure 2c). At this growth stage, the survival of the 'S' biotype at the 1x glyphosate treatment was 40% (Figure 2c). An example of the visual damage symptoms on the horseweed plants at the 18-21 leaf stage is shown in Figure 3.

After the plants bolted, the 'R' biotype plants survived all rates of glyphosate (Figure 2d, e). Similarly, glyphosate application at a 1x rate after bolting also increased the survival capability of the 'S' biotype, and it was observed that 20% of the plants were even able to survive the 2x rate (Figure 2d; e).

These results showed that the 'R' biotype was resistant to glyphosate but the level of resistance varied with growth stage. There was a probability of controlling some of the 'R' biotype horseweed at the 5-8 leaf stage with 2x and 4x rates. At later stages, even some plants of the 'S' biotype escaped the lower rates of glyphosate. This demonstrated the importance of controlling horseweed at an early stage of growth. Therefore, when using glyphosate as a post-emergent treatment, efforts should be directed to control horseweed plants very soon after emergence (before they develop more than 8 true leaves). Growers and land managers should not wait for all the horseweed plants to emerge before applying glyphosate. If the population of horseweed is to be reduced, several successive herbicide applications may have to be made to control the flushes of horseweed emergence over the growing season. An integrated weed management program should be implemented to manage the glyphosate-resistant horseweed population, including pre- and post-emergence herbicides, cultivation, and other effective methods.

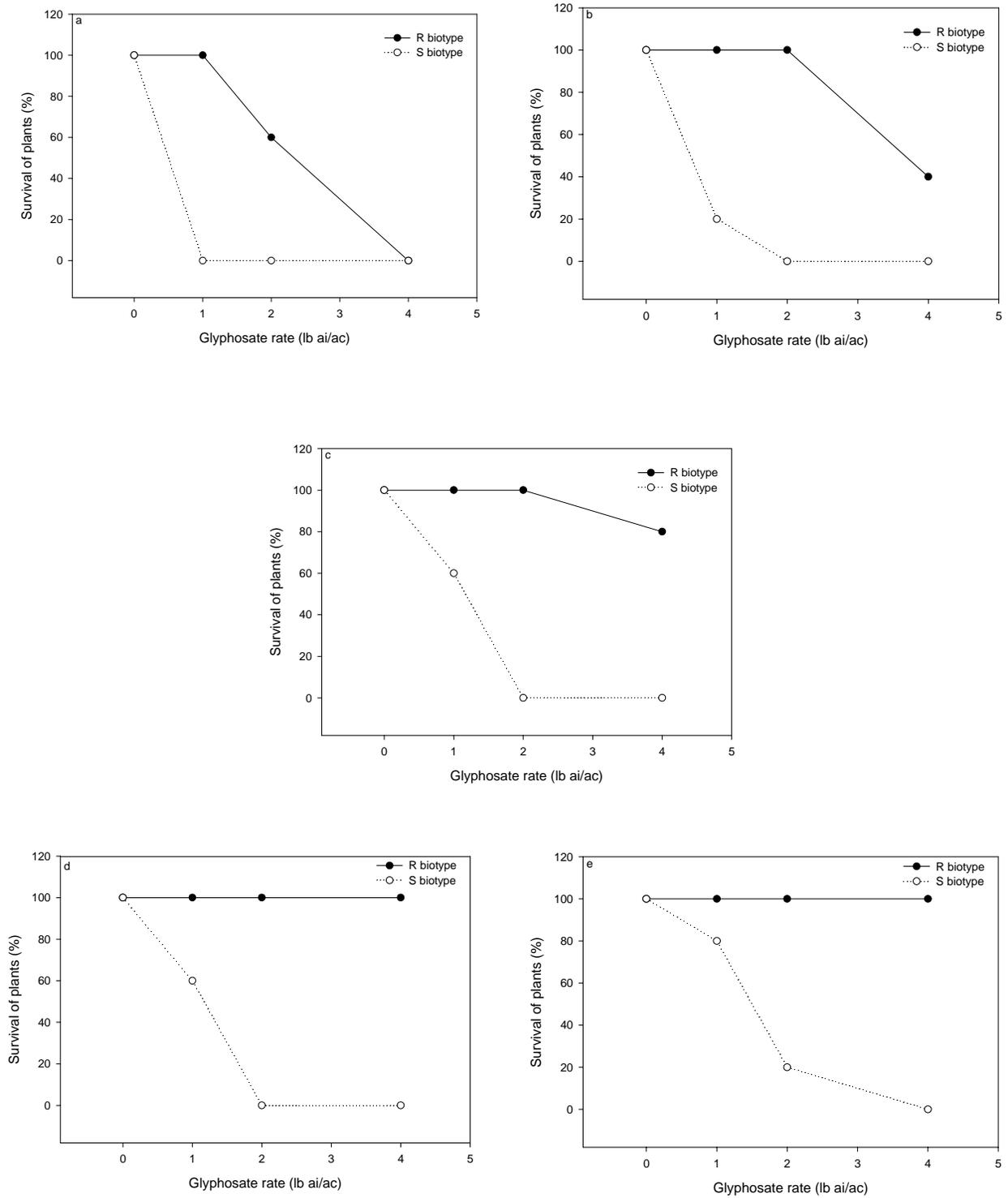


Figure 2. Horseweed plant survival under different glyphosate rates sprayed at the (a) 5-8 leaf stage, (b) 11-15 leaf stage, (c) 18-21 leaf stage, (d) bolting to 6 inch stage, and (e) 6 inch to 1 foot stage.



Figure 3. Visual damage symptoms on horseweed plants at the 18-21 leaf stage. 'R' biotype (L) and 'S' biotype (R) sprayed at 0x, 1x, 2x, and 4x rates of glyphosate (foreground to background, respectively).

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### REFLECTIVE BED MULCH BUT NOT OVER-THE-CANOPY SHADE CLOTH CONTROLS WEEDS IN FIELD-GROWN ZINNIAS

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#### Abstract

A field experiment at the Kearney Research and Extension Center (KREC) near Fresno demonstrated that reflective, silver-over-black polyethylene mulch effectively suppressed weed growth in zinnias (*Zinnia elegans* cv. Dahlia Flowered Blue Point) for cut-flower production. The mulch reduced the time needed to hand-weed plots by 85%, and mean weed biomass per plot by 97%, over the bare soil control. On the other hand, use of polymer shade cloth rated at 30% light reduction had no effect on weed management. Opaque, reflective mulches can be useful for weed management in warm-season ornamental crops in the San Joaquin Valley.

#### Introduction

Open-field production of ornamental cut flowers in California is concentrated in coastal areas, where moderate temperatures prevail year-around. Cut flower production in the San Joaquin Valley (SJV) is very limited, primarily due to more extreme cold winter and hot summer air temperatures. Zinnias are a heat-loving summer annual valued for use in ornamental borders and beds, and as cut flowers.

Previous studies in the SJV have shown increased flowering and fruiting of a variety of plants as a result of culture under enhanced light conditions provided by reflective mulches. Some of the benefits have arisen from non-chemical pest management by repulsion of herbivorous and/or viruliferous insects (Stapleton and Summers, 2002; Summers *et al*, 2004) and weed suppression. However, other studies have shown enhanced flowering and fruiting with reflective mulch in the absence of major pests (Mahmoudpour and Stapleton, 1997; Mitchell *et al*, 2000). In these cases, increased light reflecting into the plant canopy has been postulated as being the mechanism for the plant responses (Stapleton and Summers, 2002; Summers *et al*, 2004). Apart from numerous pest management benefits, the deployment of mulches and plant coverings provides soil temperature, soil moisture, and other

microenvironmental modifications which may benefit crop growth.

Most of the economically important plants which have been evaluated in conjunction with reflective mulches have been producers of edible products. There is little information available on effects in field-grown ornamental flower or foliage crops. The objectives of these experiments were to evaluate effects of reflective mulch and shade cloth on weed management in zinnias.

## Materials and Methods

Zinnia seeds (*Zinnia elegans* cv. Dahlia Flowered Blue Point) were sown in commercial potting mix and germinated in flats according to standard greenhouse conditions. Plants were transplanted to the field ca. 5 wk after seedling emergence. The reflective mulch treatment consisted of an aluminum metallized over black, reflective polyethylene film placed directly on beds. Low light treatments were established by placing black polymer shade cloth, rated at 30% light reduction, over the beds. The shade cloth was attached to wooden posts ca. 0.91 m (36 in) height above the bed level. A treatment combining the reflective soil mulch and the shade net canopy cover also was used, as was a bare soil control, to give a 2x2 factorial experimental design. Four replicate plots per treatment were used, with each replicate 6 m (20 ft.) long. Guard rows of the zinnia plants were grown around the perimeter and between the replicates of the experimental area. A single flower color series, 'Golden Dawn', was used for all data collection.

Plants were irrigated conventionally using a surface drip system, and received weekly fertilization with 17% calcium-ammonium nitrate (CAN-17) applied in the irrigation water. Although weed numbers were few on the planting beds immediately following land preparation, paraquat was applied over the entire experimental area one wk prior to transplanting, followed four days later by hula-hoeing, to eliminate all emergent weeds at the beginning of the field experiment.

Four weeks after transplanting (October 14), a two-man field crew was sent into the experiment to hand-weed each plot. Both men worked together to weed each plot, and they were timed with a stopwatch. Weeds removed from each plot were screened to remove adherent soil, placed into paper bags, and transferred to a drying oven at 70 °C. When dry, weed masses from each plot were

again screened to remove soil, then weighed to determine total weed biomass. Data were analyzed by the GLM procedure using SAS software.

## Results and Discussion

The predominant weed taxa in the experimental area were barnyardgrass (*Echinochloa crus-galli*), nutsedge (*Cyperus* spp.), pigweed (*Amaranthus* spp.), and carpetweed (*Molluga verticillata*). The mean time needed by the two field workers to remove weeds from each reflective mulch plot was 1.8 minutes, as compared to 11.8 minutes for the bare soil control, 13.1 minutes for the shade cloth, and 2.1 minutes for the combination of the reflective mulch and shade cloth. This translated into an 84.6% time reduction for the reflective mulch over the bare soil control. Factorial ANOVA gave a significant effect of reflective mulch ( $P < 0.05$ ), while the shade cloth factor, and the interaction between the reflective mulch and shade cloth, were both nonsignificant.

In terms of total weed dry weight, the reflective plastic mulch was again demonstrably more successful in inhibiting weed growth ( $P < 0.05$ ). The mean dry weed biomass per mulch plot was 8.1 g, compared to 320.6 g per plot for bare soil. This corresponded to a 97.5% reduction in weed biomass in the mulch plots, as compared to bare soil plots. Shade cloth allowed 403.7 g of weed growth per plot (25.9% greater than the bare soil control), while the combination of mulch and shade cloth allowed 9.8 g (96.9% reduction over bare soil control). There was no significant effect of shade cloth use, or for the interaction of mulch and shade cloth.

Weed populations on bare soil, whether in open sunlight or under shade cloth, were distributed over the entire bed areas. On the other hand, weed populations in reflective mulch plots were confined to the periphery of the plastic sheets and to the planting holes.

## Conclusions

This field experiment showed that opaque, silver reflective polyethylene mulch, but not shade cloth, was effective for managing weeds in zinnias for cut-flower production. The results indicated that reflective mulch can be useful for non-chemical weed management in warm-season ornamental crops in the San Joaquin Valley. Other data not shown here demonstrated that the use of reflective mulch gave increased cut-flower yields.

## Acknowledgements

This report was adapted from a presentation made by the senior author at the 2004 Annual Meeting of the California Weed Science Society. We thank Ruth Dahlquist and Albert Newton for technical assistance; Modena Seed Co., San Francisco, for donating the zinnia seeds; and the California Environmental Protection Agency, Department of Pesticide Regulation for partial funding of these studies.

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## STRATEGIES AND TACTICS FOR FUMIGATING CLAY LOAM SOILS

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It is silt and clay loam soils that best exemplify the benefit of higher vapor pressure and longer half-life associated with methyl bromide (MB). Utilizing lower vapor pressure products, we have now obtained nematode control adequate to produce a "nematode free" 2-year nursery crop within clay loam soil. This achievement requires increased attention to soil preparation and higher application rates. This report will focus on clay loam soils having no more than 19% soil moisture content within the surface 1.6 m of soil profile.

Attention to soil moisture content is paramount to successful soil fumigation, particularly when fumigants of lower vapor pressure are to be used (McKenry, 1978).

The surface 1.6 m of a loamy sand soil dried to less than 5% moisture, or a sandy loam soil dried to less than 12%, can be successfully fumigated with 370 kg/ha (330 lb/acre) 1,3-dichloropropene (1,3-D) delivered at the 50 cm depth. Note that use of a tarp or some additional surface treatment is necessary for this treatment to provide 99.99% nematode control. Treatments with 370 kg/ha 1,3-D can be comparable to those involving 370 kg/ha MB applied at the 30 cm depth followed by deployment of a plastic tarp. Pre-treatment soil ripping to a 90 cm depth generally provides adequate preparation for coarser-textured soils.

In finer-textured soils, MB at 448 kg/ha delivered at 30 cm depth and covered with a plastic tarp can provide 99.9% nematode control to 1.6 m depth in appropriately prepared soils. Equivalent nematode control with 1,3-D alone, 1,3-D plus chloropicrin (CP), or methyl iodide (MI) plus CP requires increased application rates and attention to soil moisture content. For example, if 1,3-D is to be the sole fumigant, it must be applied at 560 to 750 kg/ha where soil moistures are 12-15% or 15-19%, respectively. Just as important, for soils of 12 to 15% moisture content to receive adequate treatment they must be: 1) pre-ripped on 60 to 75 cm centers in at least one direction to the 1.3 m depth; 2) re-settled with disc and ring roller; 3) the fumigant delivery shank must have a Buessing wing mounted at two or three locations along each shank for shank trace closure; 4) fumigant delivery must be split with half being emitted at the 40-50 cm depth and half at the 60-75 cm depth; and 5) delivery shanks must be followed by a disc and ring roller device. In field settings where 15 to 19% soil moisture prevails, the conditions are as listed above except pre-ripping must reach down to 1.6 m depth. Successful combination treatments of 1,3-D with CP involve 370 kg/ha 1,3-D applied at the 40-50 cm depth plus 280 to 392 kg/ha at the 60-75 cm depth at 12-15% to 15-19% soil moisture, respectively. Successful combination treatments of MI plus CP involve replacement of the shallower 1,3-D delivery with 263 kg/ha MI.

## Implications for Growers

Adherence to the specifications listed above provides three new soil fumigation treatments that can replace MB use in clay loam soils. Although these studies were conducted to meet prevailing regulations of California nurserymen, the results have implications for orchard replant settings, as well as implications relative to reducing fumigant emissions. The CDFA nursery certification program accepted in March 2005 the

protocols for treatment of higher-moisture soils as presented in chart 1.

### **Regulatory Implications:**

Replacement of MB use in clay loam soils requires higher application rates because replacement products degrade more quickly (CP), move more slowly (1,3-D) via soil air passageways, or cannot be applied at high enough application rates (MI). California DPR does not currently suggest applications of 1,3-D in excess of 370 kg/broadcast acre. This application ceiling is based on off-gassing models involving sandy loam soils but models appropriate to finer-textured soils with appropriate soil preparation have not yet been developed.

USEPA is currently grouping soil fumigants into a single risk cup and off-gassing will become a greater issue. Our studies have shown the need for higher application rates but also introduce six application activities and each will reduce fumigant off-gassing. These include: 1) greater depths of application, 2) increase in available air passageways deep within the soil, 3) spreading of fumigant delivery points, 4) applications to soils of higher water holding capacity, 5) use of winged devices

along each shank to better fill shank traces, and 6) selection of fumigants with lower half-life. Equipment for conducting these activities is available in the US, particularly California, but the required equipment is not commonly available world-wide. Off-gassing from MB and each of its replacements should be modeled because the soil preparation indicated above could reduce emissions from clay loam soils by 2 to 5-fold.

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### **Acknowledgements**

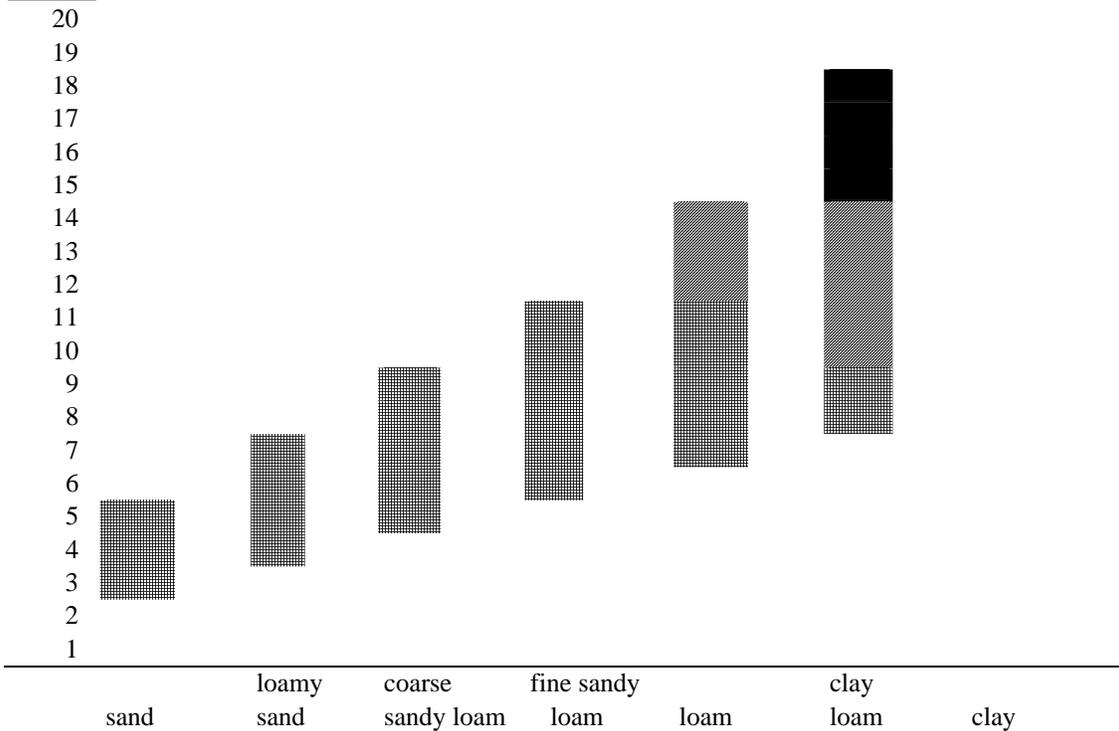
Greatly appreciated was the assistance of TriCal Inc., Hollister, CA; Stuke Nursery, Gridley, CA; Sierra Gold Nursery, Yuba City, CA; and Bob Lemos of UC Davis Pomology Dept.

**Chart 1. Fumigation requirements to obtain 27-mo-old nematode-free nursery stock.**

**Treatments:**

- 350 lb/ac MB at 10-20", tarped
- 33.7 gpa Telone II at 18-22" + 110 lb/ac MS or tarp
- 33.7 gpa Telone II at 18-22" then flip surface 12" then 15 gpa Telone II
- # 235 lb/ac MI at 18-22" depth + 150 lb/ac CP at 26-30" depth + 110 lb/ac MS
- 400 lb/ac MB at 10-20", tarped
- \*25 gpa Telone II at 18-22" + 25 gpa Telone II at 26-30" then 110 lb/ac MS or tarp
- \*33.7 gpa Telone II at 18-22" + 250 lb/ac CP at 26-30" then 110 lb/ac MS or tarp
- \*\*235 lb/ac MI at 18-22" + 300 lb/ac CP at 26-30" then 110 lb/ac MS or tarp
- \*\*33.7 gpa Telone II at 18-22" + 350 lb/ac CP at 26-30" then 110 lb/ac MS or tarp
- \*\*33.7 gpa Telone II at 18-22" + 33.7gpa at 26-30" then 110 lb/ac MS or tarp

**% H2O**



- \* must use Buessing winged shank in soil pre-ripped to 4 ft on 2 ft centers
- \*\*must use Buessing winged shank in soil pre-ripped to 5 ft on 2 ft centers
- # pluot, plum, prune and cherry scions can exhibit iodide toxicity in sandy soils

**Field preparation and assessment**

- steps 1 two years since previous perennial crop
- 2 fall-plant deeper-rooted crops such as winter wheat or oats to utilize winter rainfall
- 3 harvest by early summer to avoid green matter on field surface at fumigation time
- 4 rip soil to depth with shanks on 4 ft centers then second pass between markings  
step 4 may be substituted by a slip plow on 6 ft centers (= 3 ft between passes)
- 5 re-level and smooth as needed leaving no clods larger than 2 inch on field surface
- 6 fumigations may be applied simultaneous or MS applied first with 2-ac inch drench
- 7 MS may be applied as a drench or simultaneous with fumigation using rototiller to 5"
- 8 collect H2O %, from soil of finest texture in the block, at 1 ft increments to 5 ft depth  
note: 90 lb/ac metam potassium (at 54% ai) may be substituted for 110 lb/ac MS (42%)