SURVIVAL OF OAK ROOT SYSTEMS FOLLOWING FRILL GIRDLE HERBICIDE TREATMENT FOR OAK WILT CONTROL

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ABSTRACT.—Mechanical separation of root systems is widely used to prevent tree-to-tree vascular spread of oak wilt disease. A safe effective herbicide treatment would be valuable for this purpose in hilly, rocky, or urban settings. Three treatments were frill-girdle applied:

1) water,
2) undilutetd Garlon 3A (trichlopyr), or
3) half-strength aqueous Garlon 3A plus 24 ml per L Arsenal AC (imazapyr).

Autumn, winter, and late summer treatments were applied to white, northern red, and black oak. Root system mortality was evaluated with the vital stain 2,3,5-triphenyltetrazolium chloride. Root mortality occurred much more slowly than canopy mortality and too slowly for useful oak wilt control.

Disruption of inter-tree vascular connections via mechanical or chemical separation of grafted root systems is necessary to prevent tree-to-tree transmission of the oak wilt disease pathogen and halt expansion of oak wilt disease epicenters. Five foot deep vibratory plow line “barriers” to pathogen transmission are the currently favored tool for this purpose (for example, Blankenheim and others 2000). However, vibratory plows and 5 foot blades are not universally available and are difficult or unsafe to use in rocky soils, on steep terrain, or in urban settings. There is also concern regarding the durability of this kind of root graft barrier. For example, neither the likelihood nor the timing of root growth and re-grafting across plow lines are known.

Clearly, there is demand for an alternative tool for functional separation of grafted root systems. An herbicide treatment that safely and reliably kills oak root systems would be a particularly useful tool for halting epicenter expansion in locations inappropriate for the operation of a large tractor pulling a 5 foot blade. A variety of herbicides have been routinely used to kill unwanted trees. At least some of these herbicides can be translocated across root grafts to untreated trees, because herbicide damage to neighboring untreated trees has been observed.

The two herbicides which were most often recommended to the authors for their ability to kill red oak species were Arsenal AC (imazapyr) and Garlon 3A (trichlopyr). Because Arsenal AC is more soil-active than Garlon 3A, the possibility of non-target tree and turf damage is greater with Arsenal AC (Dr. James Miller and Max Williamson, personal communications). Aqueous mixtures of Garlon 3A and Arsenal AC have been used operationally by the USDA Forest Service, most prominently to kill unwanted hardwoods in the southern United States (Dr. James Miller and Max Williamson, personal communications). The seasons of application most often recommended were autumn (when the trees are translocating sugars downward for winter storage) and late winter (basis unknown). If herbicide treatment in the late summer (as soon as annual oak wilt symptom expression is complete) is efficaceous, epicenters could be treated when pathogen position is most accurately known.

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Our overall objective was to evaluate the effectiveness of two highly recommended herbicide treatments as potential management tools to block expansion of oak wilt epicenters where vibratory plow use is impractical. Our specific initial objectives were to:

1) identify set(s) of conditions (chemical and season of application) under which root systems of healthy susceptible trees might be quickly rendered unsuitable for service as root graft “bridges” for the expansion of oak wilt disease epicenters; and
2) determine the effectiveness of the selected treatments for killing tree root systems.

MATERIALS AND METHODS

Two hundred well-separated study trees were selected at each of three locations: Little Lost Creek Conservation Area (LLCCA) near Warrenton, MO (Warren Co.); Daniel Boone Conservation Area (DBCA) near Jonesburg, MO (Warren Co.); and Deer Ridge Conservation Area (DRCA) northwest of Hannibal, MO (Lewis Co.). The oak species under study were: *Quercus velutina* Lam. (black oak) and *Q. alba* L. (white oak) at LLCCA, *Q. velutina* and *Q. rubra* L. (northern red oak) at DBCA, and *Q. alba* and *Q. rubra* at DRCA. Each tree selected for study at each site was separated by at least 20 m from any other study tree. Larger trees were separated by greater distances from surrounding trees (see table 4 in Bruhn and others (1992) as a rough guide.

Treatments

One complete set of treatments was installed at each study site in Autumn (late-October/ early-November 1998), and another in late Winter (mid- to late-February 1999). A third complete set of treatments was installed at DBCA and LLCCA in late summer (mid-September 1999). Scheduling conflicts precluded establishment of late summer treatments at DRCA. Experimental factors were:

1) two oak species at each study site (see above);
2) two candidate herbicide treatments plus two relevant control treatments (see below); and
3) the two (DRCA) or three (LLCCA and DBCA) seasonal applications just indicated.

Ten trees per study species at each location were randomly assigned to each combination of experimental factors.

The two experimental herbicide materials used were:

1) Garlon 3A undiluted, and
2) half-strength aqueous Garlon 3A to which was added 24 ml Arsenal AC per liter of the diluted Garlon 3A.

This mixture was recommended by Max Williamson (personal communication). Both materials were prepared fresh prior to application and applied to frill girdles (FG), with cuts placed edge to edge, angled downward, and penetrating the xylem at least 1.25-cm. Trees were girdled approximately 1 m above ground level (Miller 1991). Herbicide was applied at the rate of 1 ml per 5 cm of stem circumference. The control treatments were:

1) FG, with tap water only; and
2) no FG and no herbicide.

At each study site, the same 10 trees per species served as “no frill girdle (FG) and no herbicide” reference controls in all three seasonal treatments.

It was initially intended to conclude each portion of the experiment 1 year after installation. However, observations of canopy condition and basal and stem sprouting indicated that the Autumn 1998 treatments had not yet caused adequate mortality (in light of our objective of complete tree mortality) by Autumn 1999 to warrant destructive sampling of lateral roots. Ultimately, the study was concluded in three stages: between 10-24 July 2000 at DBCA; between 11-14 June 2001 at LLCCA; and between 10-11 September 2001 at DRCA. In this manner, a single complete set of root vitality data was collected at a single point in time from each site. As a result, Summer treatments were allowed to develop for 7 and 10.5 months less, respectively, than Winter and Autumn treatments. Also, all treatments at DBCA were allowed to develop for 11 and 14.5 months less than the corresponding treatments at LLCCA and DRCA.

Tree Health Evaluation

Initial study tree condition (crown dieback, foliage transparency, and numbers of root crown and stem sprouts) was first evaluated for all study trees in October 1998 (Millers and others 1992). Study tree condition was similarly re-evaluated at study conclusion on each site; the condition of surrounding untreated trees was observed at the same time for indications of possible treatment influence. We define crown dieback as progressive branch mortality from the tip toward the bole, measured as the percentage of a tree canopy’s total two-dimensional profile which is represented by branch dieback (see Millers and others 1992). We define and measure foliage transparency as the percentage of a tree’s live canopy area through which sky is visible (see Millers and others 1992). We also report here the percentage of study trees in each treatment which supported live sprouts from the root crown during the final evaluation.

In addition, cambium vitality was evaluated on two lateral roots 5 to10 cm from their point of distinction from the root crown, either visually (based on obvious tissue discoloration or decay) or by use of the vital stain 2,3,5-triphenyltetrazolium chloride (TTC) (Parker 1953, Joslin and Henderson 1982). To evaluate a root, a block of root wood with one face representing approximately 0.5 cm by 4 cm of vascular cambium surface was immersed in a 1 percent aqueous solution of TTC in a glass screw-cap vial. The cambium surface of living samples turned red within an hour. Each evaluated root was considered to be a replicate in the sense of Glover (1991). One tree representing each species and treatment combination at DBCA was uprooted in late July 2000 to determine the health of lateral roots beyond the root crown using TTC.

Statistical Analysis

To identify the treatment factors which contributed to fastest root mortality, we used stepwise logistic regression analyses (SLR; see Sokal and Rohlf 1995), employing PROC LOGISTIC (α = 0.05; SAS/STAT System Release 6.12, SAS Institute, Inc., Cary, NC). The response variable employed was the binary (classification) variable individual root mortality. The independent variables included were: the incubation period between treatment application and evaluation (a continuously distributed variable), and treatment season (Autumn, Winter, and Summer), FG treatments (none, water, Garlon 3A, and Garlon 3A + Arsenal AC), and tree species (*Q. alba, Q. rubra*, and *Q. velutina*).

RESULTS

All treatments at DBCA (*Q. rubra* and *Q. velutina*) were evaluated in July 2000, after 20.5 months, 17 months, and 10 months incubation, respectively, of the Autumn, Winter, and Summer applications (table 1). The canopies of all trees treated with Garlon (except one *Q. velutina* treated with Garlon in the summer) or the Garlon plus Arsenal mixture appeared dead. The canopies of trees which received the FG plus water treatment averaged at least 48 percent dieback and 64 percent canopy transparency, whereas untreated “control” *Q. rubra* and *Q. velutina* trees averaged, respectively, no more than 17 and 22 percent dieback and 12 and 18 percent canopy transparency. Of the trees receiving FG plus water, Garlon, and Garlon plus Arsenal, respectively, 68 percent, 25 percent, and 30 percent supported live sprouts at the root crown in July 2000.

All treatments at LLCCA (*Q. alba* and *Q. velutina*) were evaluated in June 2001, after 31.5 months, 28 months, and 21 months development, respectively, of the Autumn, Winter, and Summer applications (table 2). The canopies of all trees treated with Garlon or the Garlon plus Arsenal mixture appeared dead. The canopies of trees which received the FG plus water treatment averaged at least 88 percent dieback and 83 percent canopy transparency, whereas untreated “control” *Q. alba* and *Q. velutina* trees averaged, respectively, no more than 18 and 28 percent dieback and 17 and 26 percent transparency. Of the trees receiving FG plus water, Garlon, and Garlon plus Arsenal, respectively, 47 percent, 18 percent, and 23 percent supported live sprouts at the root crown in June 2001.

All treatments at DRCA (*Q. alba* and *Q. rubra*) were evaluated between mid-September and the end of October 2001, after approximately 35 months and 31.5 months incubation, respectively, of the Autumn and Winter applications (table 3). The canopies of all trees treated with Garlon or the Garlon plus Arsenal mixture appeared dead. The canopies of trees which received the FG plus water treatment averaged at least 92 percent dieback and 92 percent transparency, whereas untreated “control” *Q. alba* and *Q. rubra* trees averaged, respectively, no more than 25 and 30 percent dieback and 14 and 26 percent transparency. Of the trees receiving FG plus water, Garlon, and Garlon plus Arsenal, respectively, 35 percent, 8 percent, and 11 percent supported live sprouts at the root crown in September-October 2001.

Lateral Root Survival

Overall, both, one, or neither of the two sampled roots were dead, respectively, on 44 percent, 15 percent, and 41 percent of the study trees at the time of their evaluation. Root mortality was incomplete in all but 3 of the 48 combinations of season, chemical, and tree species. All treatment combinations incubated 28 months or less revealed substantial root survival (tables 1, 2, and 3). All *Q. velutina* roots in the Autumn Garlon treatment at LLCCA were dead after 31.5 months incubation, but 25 percent of corresponding roots at DBCA remained alive after 20.5 months incubation. All *Q. rubra* roots in the Autumn Garlon treatment at DRCA were dead after 35 months incubation, but 20 percent of corresponding roots at DBCA remained alive after 20.5 months incubation. All *Q. rubra* roots in the Winter Garlon treatment at DRCA were dead after 31.5 months incubation, but 70 percent of corresponding roots at DBCA remained alive after 17 months incubation.

Table 1.—*Effects of frill girdle herbicide treatments on lateral root survival, root crown sprouting, and canopy health for* Quercus rubra *and* Q. velutina *at Danial Boone Conservation Area (DBCA)*

Season	Elapsed time[1]	Treatment[2]	Species	DBH		Live sprouts	Live roots	Crown dieback		Foliage transparency	
	months			*cm*	*(SD)*	*%*	*%*	*%*	*(SD)*	*%*	*(SD)*
Autumn	20.5	Tap Water	*Q. rubra*	20	(5)	90	65	94	(17)	92	(27)
			Q. velutina	22	(4)	50	40	90	(20)	86	(29)
		Garlon 3A	*Q. rubra*	22	(7)	0	20	100	(0)	100	(0)
			Q. velutina	24	(6)	0	25	100	(0)	100	(0)
		G+Arsenal	*Q. rubra*	21	(4)	40	60	100	(0)	100	(0)
			Q. velutina	25	(7)	10	25	100	(0)	100	(0)
Winter	17	Tap Water	*Q. rubra*	22	(10)	80	75	100	(0)	100	(0)
			Q. velutina	24	(3)	50	40	99	(2)	88	(26)
		Garlon 3A	*Q. rubra*	23	(6)	60	70	100	(0)	100	(0)
			Q. velutina	24	(8)	60	60	100	(0)	100	(0)
		G+Arsenal	*Q. rubra*	23	(8)	40	80	100	(0)	100	(0)
			Q. velutina	26	(7)	20	85	100	(0)	100	(0)
Summer	10	Tap Water	*Q. rubra*	21	(6)	90	100	48	(39)	64	(32)
			Q. velutina	27	(9)	50	100	60	(42)	64	(38)
		Garlon 3A	*Q. rubra*	22	(10)	20	80	100	(0)	100	(0)
			Q. velutina	22	(4)	10	100	100	(2)	92	(24)
		G+Arsenal	*Q. rubra*	22	(4)	20	100	100	(0)	100	(0)
			Q. velutina	22	(4)	50	100	100	(0)	100	(0)
Control		None	*Q. rubra*	22	(5)	20	100	17	(8)	12	(4)
			Q. velutina	23	(6)	0	100	22	(11)	18	(5)

[1]Elapsed time: The elapsed time between chemical application and root sampling.
[2]Treatment: All chemicals were applied to edge to edge frill girdles at the rate of 1.0 ml per 5.0 cm tree circumference - Garlon 3A, undiluted; G+Arsenal, half-strength Garlon 3A to which was added 24 ml Arsenal AC per liter of diluted Garlon 3A.

Treatment Factor Effectiveness

As expected, SLR indicated that the elapsed time between treatment and final evaluation (ranging between 10 and 35 months in this study) was directly related to the level of root mortality observed (table 4). Autumn treatment was more effective than Winter or Late Summer treatment. All FG treatments were more effective than no treatment at all. The FG plus water treatment was less effective than either of the herbicide treatments, and the FG plus Garlon treatment was most effective. Root mortality occurrred more slowly in *Q. alba* and *Q. rubra* than in *Q. velutina*.

DISCUSSION AND CONCLUSIONS

Barriers to root graft transmission of the oak wilt pathogen must separate all infected roots (inside the barrier) from all healthy root systems outside the barrier. They are expensive and time-consuming to install. In residential and park settings, the installation and effectiveness of barriers receive close public scrutiny, and the forest health professional's credibility is at stake.

To accomplish an effective barrier, one must recognize that by the time a tree at the epicenter's edge is displaying symptoms, the pathogen has often already reached the root systems of trees to which the symptomatic tree is grafted. Therefore, barriers need to be placed outside non-symptomatic trees which are close enough to symptomatic trees to be grafted directly to them (Bruhn and others 1992). In other words, an immediately effective barrier (for example, one established with a vibratory plow) must be established just beyond the equivalent of one year's disease spread outside the visible epicenter margin.

Proper barrier placement, recognizing that some non-symptomatic trees at the margin of an epicenter are already infected, creates the impression that at least one tier of non-symptomatic trees is being "sacrificed". In the process of modeling the expansion of oak wilt epicenters in northern pin oak stands (*Q. ellipsoidalis* E. J. Hill) growing on sandy soils in Michigan's Upper Peninsula (Bruhn and others 1992), radial expansion of up to 12.5 m (between two trees with combined dbh of 75.7 cm) was documented.

Table 2.—*Effects of frill girdle herbicide treatments on lateral root survival, root crown sprouting, and canopy health for* Quercus alba *and* Q. velutina *at Little Lost Creek Conservation Area (LLCCA)*

Season	Elapsed time[1]	Treatment[2]	Species	DBH		Live sprouts	Live roots	Crown dieback		Foliage transparency	
	months			*cm*	*(SD)*	*%*	*%*	*%*	*(SD)*	*%*	*(SD)*
Autumn	31.5	Tap Water	*Q. alba*	22	(8)	50	80	88	(27)	83	(36)
			Q. velutina	29	(10)	30	5	100	(0)	100	(0)
		Garlon 3A	*Q. alba*	18	(7)	0	15	100	(0)	100	(0)
			Q. velutina	24	(10)	11	0	100	(0)	100	(0)
		G+Arsenal	*Q. alba*	19	(6)	10	45	100	(0)	100	(0)
			Q. velutina	27	(10)	0	20	100	(0)	100	(0)
Winter	28	Tap Water	*Q. alba*	15	(3)	60	60	100	(0)	100	(0)
			Q. velutina	23	(7)	20	15	100	(0)	100	(0)
		Garlon 3A	*Q. alba*	19	(10)	30	40	100	(0)	100	(0)
			Q. velutina	25	(8)	11	30	100	(0)	100	(0)
		G+Arsenal	*Q. alba*	18	(7)	10	50	100	(0)	100	(0)
			Q. velutina	23	(5)	33	35	100	(0)	100	(0)
Summer	21	Tap Water	*Q. alba*	18	(8)	40	80	100	(0)	100	(0)
			Q. velutina	19	(3)	80	65	99	(3)	98	(6)
		Garlon 3A	*Q. alba*	21	(8)	30	60	100	(0)	100	(0)
			Q. velutina	24	(10)	22	55	100	(0)	100	(0)
		G+Arsenal	*Q. alba*	18	(9)	0	55	100	(0)	100	(0)
			Q. velutina	25	(9)	0	35	100	(0)	100	(0)
Control		None	*Q. alba*	19	(6)	10	100	18	(11)	17	(8)
			Q. velutina	21	(5)	10	100	28	(27)	26	(27)

[1]Elapsed time: The elapsed time between chemical application and root sampling.
[2]Treatment: All chemicals were applied to edge to edge frill girdles at the rate of 1.0 ml per 5.0 cm tree circumference - Garlon 3A, undiluted; G+Arsenal, half-strength Garlon 3A to which was added 24 ml Arsenal AC per liter of diluted Garlon 3A.

Table 3.—*Effects of frill girdle herbicide treatments on lateral root survival, root crown sprouting, and canopy health for* Quercus alba *and* Q. rubra *at Deer Ridge Conservation Area (DRCA)*

Season	Elapsed time[1]	Treatment[2]	Species	DBH		Live sprouts	Live roots	Crown dieback		Foliage transparency	
	months			*cm*	*(SD)*	*%*	*%*	*%*	*(SD)*	*%*	*(SD)*
Autumn	35	Tap Water	*Q. alba*	20	(3)	50	30	100	(0)	100	(0)
			Q. rubra	26	(13)	40	30	100	(0)	100	(0)
		Garlon 3A	*Q. alba*	19	(6)	10	15	100	(0)	100	(0)
			Q. rubra	25	(8)	11	0	100	(0)	100	(0)
		G+Arsenal	*Q. alba*	22	(4)	10	10	100	(0)	100	(0)
			Q. rubra	22	(6)	20	15	100	(0)	100	(0)
Winter	31.5	Tap Water	*Q. alba*	27	(8)	50	95	92	(18)	92	(24)
			Q. rubra	21	(6)	70	60	94	(19)	92	(25)
		Garlon 3A	*Q. alba*	27	(10)	10	40	100	(0)	100	(0)
			Q. rubra	22	(13)	0	0	100	(0)	100	(0)
		G+Arsenal	*Q. alba*	23	(9)	10	30	100	(0)	100	(0)
			Q. rubra	19	(8)	10	10	100	(0)	100	(0)
Control		None	*Q. alba*	28	(12)	0	100	25	(11)	13	(3)
			Q. rubra	22	(13)	0	95	30	(28)	26	(28)

[1]Elapsed time: The elapsed time between chemical application and root sampling.
[2]Treatment: All chemicals were applied to edge to edge frill girdles at the rate of 1.0 ml per 5.0 cm tree circumference - Garlon 3A, undiluted; G+Arsenal, half-strength Garlon 3A to which was added 24 ml Arsenal AC per liter of diluted Garlon 3A.

Table 4.—*Results of stepwise logistic regression to identify factors associated with root mortality*

	EXPLANATORY VARIABLES			PREDICTIVE EVALUATION[3]		
Intercept	**Name[1]**	**Parameter estimate**	**Pr > Wald Chi-square[2]**	**Percent Concordant**	**Percent Discordant**	**No. pairs**
- 3.3682			0.0001	87.1	12.1	369413
	Elapsed Time	0.1533	0.0001			
	Autumn	0.6079	0.0003			
	FG + Water	-0.5612	0.0029			
	FG + Garlon 3	0.5750	0.0025			
	Control	-5.1045	0.0001			
	Q. alba	-1.3066	0.0001			
	Q. rubra	-0.4547	0.0181			

[1]Variable: Elapsed time: The elapsed time between chemical application and root sampling; FG, edge to edge frill girdle treatment; Control, nontreated.
[2]The significance test used by PROC LOGISTIC is based on the Wald statistic (Sokal and Rohlf 1995).
[3]No. Pairs represents the product (Observed) * (Total - Observed), the number of possible pairwise combinations of sampled roots in which one sampled root was dead and the other remained alive, respectively. For each sampled root, SLR assigned a probability, p, that the sampled root would be dead. 'Percent Concordant' is the percentage of pairs for which the larger value of p was associated with the dead root. 'Percent Discordant' is the percentage of pairs for which the larger value of p was associated with the surviving root.

One should anticipate annual expansion over greater distances around epicenters in stands of larger trees, and over shorter distances in stands growing on heavier soils (Bruhn and others 1992). Placement of a barrier which is not immediately effective (for example, a band of herbicide-treated trees) should take into account the epicenter expansion anticipated during the time required for the barrier to become effective. This would result in the sacrifice of additional land area and the healthy trees growing there.

A variety of herbicides has been used operationally in efforts to create barriers to root graft spread of oak wilt. Frill girdle application with Garlon 3A was used rather than basal stem spray application of Garlon 4 to achieve greater effectiveness on large diameter stems. The Specimen Label for Dow AgroSciences' Forestry Garlon 4 recommends use of this product as a basal bark treatment on stems less than 6 inches in basal diameter.

Frills were created with hacks placed edge-to-edge rather than the more standard spacing of 3 inches between hacks (Miller 1991), to accomplish the most rapid and complete treatment effect. Though extremely laborious, frills cut at a downward angle into the wood have the advantage of trapping the applied herbicide, whereas an unacceptable portion of herbicide applied to a chainsaw frill might be washed out by rain into the soil.

Tordon RTU is often suggested as a good candidate for causing rapid root death. However, Bruhn and Haugen (unpublished) have each observed that Tordon RTU applied via cut frill to create barrier zones of trees in late summer failed to halt the radial expansion of oak wilt epicenters in the Upper Peninsula of Michigan and in northeast Iowa, respectively. In each case, the disease expanded beyond the barrier of treated trees in a single year. In addition, it was observed that the application of chainsaw frills can result in the creation of hazard trees, as the structural integrity of the treated tree is compromised.

In a small trial in Iowa (Walkowiak, Bruhn and Haugen, unpublished), Tordon treated trees sprouted and Garlon 4 treated trees did not, suggesting that a trichlopyr herbicide might be more effective than Tordon RTU in killing the root crown. In the larger study described in this paper, the canopies of trees treated with Garlon 3A or the Garlon 3A plus Arsenal mixture all appeared dead 35 months after late Autumn treatment; however, some of the trees of each species still supported live sprouts at the root crown. Canopy mortality occurred well in advance of root mortality, and absence of sprouts at the root crown is not a dependable indication of root system mortality (tables 1, 2 and 3).

Our study is the first to evaluate the timing of tree root mortality following herbicide treatments in forestry applications. The failure of herbicides to cause rapid root death is disappointing. If herbicides require 3 years to reliably render the root systems of treated trees disfunctional for root graft transmission of the pathogen, then the herbicide barrier needs to be placed approximately 4 years' radial spread beyond the currently visible epicenter margin. This could result, for example, in the "sacrifice" of a belt of non-infected (as well as non-symptomatic) trees 50 m deep rather than 12.5 m deep as might be the case for a barrier zone constructed with a vibratory plow. In most cases, a herbicide barrier with this type of location requirement would be considered too inefficient.

We have tested two highly recommended herbicide treatments using a vital staining technique which can easily be used to evaluate other candidate herbicides. No herbicide treatment has yet been demonstrated to provide root death quickly enough to ensure the discontinuity of root systems necessary to reliably contain oak wilt disease epicenters. However, the extent of root system mortality required to effectively block pathogen transmission is not known. Considering the costs involved, establishment of barriers at a distance sufficient to permit herbicides to take adequate effect seems currently unacceptable.

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