Cyclanilide Induces Lateral Branching in Sweet Cherry Trees

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Abstract. A new bioregulator, cyclanilide (CYC, Bayer Environmental Science, Research Triangle Park, NC 27709), was compared with a proprietary formulation of 6-benzyladenine and gibberellins A₄ and A₇ [Promalin (PR), Valent BioSciences, Walnut Creek, Calif.] for branching effects on sweet cherry trees. CYC stimulated the formation of lateral shoots on current-season's shoot growth under both orchard and nursery conditions. In the nursery CYC was as effective or better for feathering compared to PR in all cherry cultivars tested. There were no synergistic effects of CYC/PR tank mixes on feather development. Crotch angles of induced feathers were not different from the angles of feathers that formed spontaneously. The growth of CYC-induced feathers was sufficient to produce acceptable quality feathered trees. Trunk caliper of nursery trees was either not affected or reduced to a very minimal degree. CYC is effective for lateral branch induction in sweet cherry, especially in the nursery. Chemical names used: 1-(2,4-dichlorophenylaminocarbonyl)-cyclopropane carboxylic acid (cyclanilide); N-(phenylmethyl)-1H-purine-6-amine + gibberellins A₄ and A₇ (Promalin); polyoxyethylenepolypropoxypropanol, dihydroxypropane, 2-butoxyethanol (Regulaid).

The rapid development of lateral branching in apple trees favors early and increased yields (Ferree and Rhodus, 1987; Preston, 1968; Quinlan, 1978, 1981; Quinlan and Preston, 1978; van Oosten, 1978, 1981; Wertheim 1978). For this reason apple trees with well-developed sylleptic shoots (feathers) from the nursery are preferred for high-density plantings. Much research has been conducted on stimulation of feathering in apple nursery trees (Elfving and Visser, 2005). In contrast, little research has been carried out on the induction of sylleptic shoot (feather) formation in sweet cherry trees. Many bioregulator products that stimulate lateral shoot development in apple or pear are ineffective when applied to sweet cherry (Cody et al., 1985b; Larsen, 1979; Plich and Basak, 1978; Quinlan and Preston, 1973). Application of the cytokinin 6-benzyladenine (BA) with or without gibberellic acid isomers GA₄ and GA₇ (GA₄₊₇) improves feather formation in sweet cherry trees in the nursery (Cody et al., 1985a; Hrotko et al., 1999; Neri et al., 2003; Wustenberghs and Keulemans, 1999), although cultivars display large differences in their response to such treatments (Wustenberghs and Keulemans, 1999).

Sweet cherry trees exhibit strong apical dominance, naturally producing a vigorous, upright growth habit with limited lateral branching in young trees (Miller, 1983;

Veinbrants and Miller, 1981). Heavy pruning is often employed to induce lateral branch development, but this practice stimulates vigor and delays economic returns. The strength of apical dominance in apple has been related to the amount of auxin in shoot tips and its movement below the tip area (Abbas, 1978; Baldini et al., 1973); it is likely that a similar phenomenon is active in sweet cherry as well. Recently, the bioregulator cyclanilide (CYC), which appears to interfere with auxin transport and action (Pederson et al., 1997), was shown to induce feather development in nursery- and orchard-grown apple trees (Elfving and Visser, 2005). The research reported here was undertaken to evaluate the potential of CYC for inducing feather development in sweet cherry trees in the orchard and nursery and to compare its effects on branch development with those of a proprietary mixture of 6-benzyladenine and gibberellic acid isomers GA₄ and GA₇ [Promalin (PR)] .

Materials and Methods

Five experiments were conducted between 2002 and 2003. All trials used randomized complete-block designs with at least 4 replications. Proprietary formulations of CYC (Bayer Environmental Science, Research Triangle Park, N.C.) and BA plus GA₄₊₇ (PR, Valent BioSciences, Walnut Creek, Calif.) were used. All bioregulator treatments were supplemented with 0.1% v/v Regulaid (Kalo, Inc., Overland Park, Kan.). Orchard plots were sprayed to runoff with a Nifty motorized hydraulic sprayer and handgun; the nursery trials were carried out using a manually operated Solo backpack sprayer fitted with two spray heads that applied a dilute spray to the upper half of each

nursery tree. The orchard trees had no flowers or fruit the year of treatment. All bioregulator concentrations are expressed in terms of the active ingredient(s). The experiments are described below.

Experiment 1, 2002, Monitor, Wash. Applications of CYC and/or PR were made to single-tree plots in six randomized blocks of 'Bing'/Mazzard sweet cherry trees planted in a commercial orchard at a spacing of 6.1 × 6.1 m in 2000. In Spring 2002 the trees were headed to encourage strong multiple leader development and were in vigorous shoot growth at the time of treatment. CYC at 50, 100, or 200 mg·L⁻¹ was applied on 20 May (terminal shoots 27.0 ± 0.4 cm). On the same date, one additional tree in each block was treated with PR (500 mg·L⁻¹) either alone or tank-mixed with CYC at 50 or 100 mg·L⁻¹. Control trees were unsprayed. After shoot growth was completed, three vigorous, upright shoots that grew in 2002 were selected on each test tree and the length of each shoot measured from its base to its tip. The length and crotch angle of each sylleptic shoot (feather) induced on each of these current-season's shoots were measured. The distance from the base of each 2002 shoot to the first induced feather was also measured.

Experiment 2, 2003, Bray's Landing, Wash. Applications of CYC and/or PR were made to single-tree plots in four randomized blocks of 'Rainier'/Mazzard sweet cherry trees planted in a commercial orchard at a spacing of 2.7 × 4.9 m in 2002. The vigorous, upright shoots that developed in 2002 were each headed in early Spring 2003 before growth began to produce the steep-leader multiple-leader training system. The following treatments were applied either once to single-tree plots on 21 May [newly developing terminal shoots 26.8 ± 0.7 cm] or twice (21 May and again 1 July (terminal shoots 101.1 ± 1.7 cm)): CYC 50 or 100 mg·L⁻¹, PR 250 mg·L⁻¹, CYC 50 or 100 mg·L⁻¹ + PR 250 mg·L⁻¹ as a tank-mix (total of 11 treatments including control). Control trees were unsprayed. After shoot growth was completed, the newly developed terminal shoot from each of three headed 2002 shoots was selected and its length determined. The length and crotch angle of all feathers that were induced on each of the 2003 terminal shoots were measured. The distance from the base of each 2003 shoot to the first induced feather was also measured.

Spontaneous formation of feathers in low numbers on sweet cherry trees occurs frequently in the nursery. In the nursery trials, the occasional feathers formed well below the approximate height of the central leader at treatment were already growing at the time of treatment and were ignored.

Experiment 3, 2002, Quincy, Wash. CYC (50 or 100 mg·L⁻¹), PR (250 mg·L⁻¹), or tank-mixes of PR with each of the CYC concentrations were applied to 10-tree plots of nursery 'Bing' and 'Lapins'/Mazzard sweet cherry trees in four randomized blocks each on 5 July. 'Bing' trees averaged about 70 cm from bud union to shoot tip at the time of treatment while 'Lapins' trees averaged about 20 cm

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Table 1. Effects of cyclanilide (CYC) and/or Promalin (PR) applications on lateral branch (feather) formation in third-leaf 'Bing'/Mazzard sweet cherry trees in the orchard (Expt. 1, 2002) (Monitor, Wash.).

Treatment (mg·L ⁻¹) ^z	Shoot length (cm)	Feathers/shoot		Mean feather length (cm)	Crotch angle (°)
		No.	No./cm shoot length (×100) ^y		
Control	207 a ^x	0.8c	0.4c	40 a	62 a
CYC 50	195 a	7.1 ab	3.6 a	30 b	42 bc
CYC 100	190 a	5.8 ab	2.9 ab	30 b	47 b
CYC 200	195 a	5.7 ab	2.9 ab	28 c	47 b
PR 500	206 a	2.2 c	1.1 c	40 a	50 b
CYC 50 + PR 500	202 a	5.0 b	2.4 b	36 ab	44 bc
CYC 100 + PR 500	194 a	7.8 a	4.0 a	33 bc	33 c

^zDilute applications with hydraulic sprayer 20 May 2002 (terminal shoots 27.0 ± 0.4 cm).

^yActual shoot no. per cm ratio values multiplied by 100 before analysis to facilitate presentation.

^xMean separation in columns by Waller-Duncan Bayesian k ratio test following significant F test ($P \leq 0.05$).

taller. Control trees were unsprayed. The trees were removed from the nursery in November 2002, bundled by replication and stored for further evaluation. For each cultivar, three representative trees from each replication were selected for detailed measurements. The following measurements were taken on each tree: 1) length of central leader from bud union to tip, 2) total number of feathers (any lateral shoot >10 cm in length) originating near or above the approximate height of the central leader at the time of treatment, 3) length of each feather, 4) crotch angle of each feather, and 5) distance from bud union to the first induced feather.

Experiment 4, 2003, Quincy, Wash. CYC (100 mg·L⁻¹) with or without PR (500 mg·L⁻¹) as a tank-mix was applied to 15-tree plots of nursery trees of 'Bing' and 'Skeena'/Mazzard sweet cherry trees in four randomized blocks on 16 June, when the newly developing central-leader shoot tips were about 60 cm above the bud unions. Control trees were unsprayed and another group of 15 trees in each block was treated with PR (500 mg·L⁻¹) on the same date. The trees were removed from the nursery in November 2003, bundled by replication, and stored for further evaluation. Ten representative trees were selected from each replication for growth measurements, which included 1) caliper 10 cm above the bud union, 2) length of the central-leader shoot from the bud union to the tip, 3) the number of feathers (any lateral shoot >10 cm in length) originating near or above the approximate height of the central leader at the time of treatment, and 4) the distance from the bud union to the first induced feather. On three of the 10 trees additional measurements were made of 1) the length of each feather and 2) the crotch angle of each feather. All 'Skeena' trees were topped in the field before digging and storing.

Experiment 5, 2003, Quincy, Wash. CYC (50 or 100 mg·L⁻¹) with or without PR (500 mg·L⁻¹) as a tank-mix was applied to 15-tree plots of nursery trees of 'Lapins'/Mazzard sweet cherry trees in four randomized blocks on 16 June, when the newly developing central-leader shoot tips were about 90 cm above the bud unions. Control trees were unsprayed and another group of 15 trees in each block was treated with PR (500 mg·L⁻¹) on the same date. The trees were removed from the nursery in November 2003, bundled by replication, and

stored for further evaluation. Measurements were determined as described for Expt. 4. Trees were not topped before storage.

Analyses of variance, regression and orthogonal contrasts were used to assess the significance of treatments and, in the appropriate trials, the relation of response to bioregulator concentration. Mean values were separated where appropriate with analysis of variance or the Waller-Duncan Bayesian k-ratio test ($P \leq 0.05$). Analyses of regression assessed the presence of significant linear and curvilinear effects of bioregulator concentrations (Elfving, 1990; Snedecor and Cochran, 1980). Statistical analyses were performed using the General Linear Models procedure of the Statistical Analysis System program package (SAS Institute, Cary, N.C.).

Results

Experiment 1. The final length of the new shoots that grew in 2002 was not affected by treatment (Table 1). CYC induced about 6-fold

Fig. 1. Experiment 1 ('Bing'/Mazzard, orchard, 2002). (a) Control shoot growth from headed leaders in 2002; (b) CYC 100 mg·L⁻¹ applied 20 May; (c) PR 500 mg·L⁻¹ applied 20 May; (d) CYC 100 mg·L⁻¹ + PR 500 mg·L⁻¹ tank mix applied 20 May. Note the cluster of feathers located near the base of each 2002 shoot in b and d, illustrating the temporary release of newly formed buds from apical dominance due to CYC treatment.

Table 2. Effects of cyclanilide (CYC) and/or Promalin (PR) applications on lateral branch (feather) formation in second leaf 'Rainier'/Mazzard sweet cherry trees in the orchard (Expt. 2, 2003) (Bray's Landing, Wash.).

Treatment (mg·L ⁻¹) ^z	Shoot length (cm)	Feathers/shoot		Mean feather length (cm)	Crotch angle (°)
		No.	No./cm shoot length (×100) ^y		
Control	207 ab ^x	1.2 e	0.5 f	40 abc	99 a
CYC 50, 1×	207 ab	6.8 abcd	3.2 abcde	32 abc	62 bc
CYC 50, 2×	174 ab	7.6 abc	4.5 ab	31 abc	54 bc
CYC 100, 1×	197 ab	8.2 ab	4.1 abc	29 bc	47 bc
CYC 100, 2×	157 b	3.8 cde	2.1 cdef	27 c	44 c
PR 250, 1×	204 ab	3.2 de	1.6 ef	45 a	70 b
PR 250, 2×	213 a	4.2 cde	1.9 def	44 ab	70 b
CYC 50 + PR 250, 1×	173 ab	4.9 bcde	2.7 bcde	30 abc	39 c
CYC 50 + PR 250, 2×	201 ab	7.7 abc	3.8 abcd	41 abc	55 bc
CYC 100 + PR 250, 1×	202 ab	9.1 a	4.6 ab	29 bc	46 bc
CYC 100 + PR 250, 2×	184 ab	9.2 a	5.0 a	30 abc	53 bc
Orthogonal contrasts					
Control vs. all treatments	NS	*	*	*	NS
All CYC vs. all PR	*	*	*	*	*
Single application vs. double application	NS	NS	NS	*	*
Single applicatio CYC 50 vs. 100 mg·L ⁻¹	NS	*	*	NS	NS

^zDilute applications with hydraulic sprayer 21 May 2003 (terminal shoots 26.8 ± 0.7 cm) and for double applications 1 July 2003 (terminal shoots 101.0 ± 1.7 cm).

^yActual shoot no. per cm ratio values multiplied by 100 before analysis to facilitate presentation.

^xMean separation in columns by Waller-Duncan Bayesian k ratio test following significant F test ($P \leq 0.05$).

NS, *Nonsignificant or significant at $P \leq 0.05$.

more new lateral (syllaptic) shoots (feathers) and comparably increased their density on the current-season's developing shoots than originated naturally, regardless of the concentration used, although some phytotoxicity to foliage was observed at the highest concentration. PR alone did not produce greater development of feathers than on untreated trees and did not improve the feathering response to CYC

when tank-mixed (Fig. 1). The distance from the shoot base to the first feather was shorter for CYC treatments and the CYC 100 mg·L⁻¹ + PR 500 mg·L⁻¹ treatment. All bioregulator treatments resulted in shorter feathers than in untreated trees, where very low incidence of syllaptic shoots coincided with the longest mean length. However, induced feathers were well-developed by the end of the growing

season. Crotch angles were unaffected by any treatment.

Experiment 2. There were a few differences in the lengths of the new terminal shoots developed in 2003 but no treatment produced different lengths compared to shoots on control trees (Table 2). The number of induced feathers and their density on the 2003 shoots were both increased substantially by CYC treatment, except for CYC 100 mg·L⁻¹ applied twice. PR treatment did not improve feathering beyond controls and did not improve the branching response to CYC when tank-mixed. No advantage in feather number or growth and development resulted from double applications of CYC. Mean feather length was shorter for all treatments, including those that did not improve branching. However, induced feathers were well-developed by the end of the growing season. While there were some statistically significant differences in crotch angle, no treatment produced any effects compared to controls and differences were minor.

Experiment 3. Final central leader length in nursery 'Bing' trees was not affected by any bioregulator treatment in 2002 (Table 3). Feathering was improved in direct proportion to CYC concentration; PR also increased feather development but its effect was not interactive with CYC. In the absence of PR, CYC produced feathers at a lower height on the shoots than occurred naturally, but when PR was included, induced feathers originated at a similar height regardless of CYC concentration. There were no effects of bioregulators on feather crotch angle. Mean feather length was not affected by either product. Leader length was slightly reduced by CYC in 'Lapins' but unaffected by PR (Table 4). As in 'Bing', feather production in 'Lapins' was increased by CYC in proportion to concentration. The distance to first induced feather was shorter in the absence of PR, but there were no effects of CYC or PR on mean feather length or crotch angle. 'Lapins' is reported to be a difficult-to-branch cultivar (Wustenberghs and Keulemans, 1999). PR did not improve feathering or affect feather growth in 'Lapins' in 2002.

Experiment 4. In 2003, feather development in both 'Bing' and 'Skeena' was increased by both CYC and PR (Tables 5 and 6, Fig. 2). CYC reduced the height to first feather and mean feather length in both cultivars and reduced total leader length slightly in 'Bing', but had only minor or no effects on trunk caliper or branch crotch angle in either cultivar.

Experiment 5. CYC increased feather development on 'Lapins' in 2003 in the presence or absence of PR (Table 7, Fig. 3). CYC produced minor effects on trunk caliper, leader length, height to feather and crotch angle; CYC had no effect on mean feather length despite the increase in number. The only effect of PR was to produce a small increase in feather development.

Discussion

Although sweet cherry trees may produce a small amount of syllaptic shoot (feather) development spontaneously, particularly in the

Table 3. Effects of cyclanilide (CYC) and/or Promalin (PR) applications on feather formation in 'Bing' / Mazzard sweet cherry trees in the nursery (Expt. 3, 2002) (Quincy, Wash.).

Growth regulator (mg·L ⁻¹) ^z	Leader length (cm)	No./ shoot	Induced feathers		Mean length (cm)	Crotch angle (°)
			Union to first induced feather (cm)			
			−PR	+PR		
CYC						
0	175	1.2	113	85	43	37
50	178	2.6	83	84	48	37
100	167	4.6	81	84	41	38
Significance						
CYC linear	NS	****	**	NS	NS	NS
CYC quadratic	NS	NS	*	NS	NS	NS
PR						
0	174 a ^y	2.1 b	88 a	---	47 a	38 a
250	173 a	4.2 a	---	85 a	42 a	37 a
Interaction						
CYC × PR	NS	NS	*		NS	NS

²Dilute applications with hydraulic sprayer 5 July 2002 (central leader about 70 cm in length).

³Mean separation in columns by F test ($P \leq 0.05$).

NS,*,**,***,****Nonsignificant or significant analysis of regression at $P \leq 0.05$, 0.01, 0.001, or 0.0001.

Table 4. Effects of cyclanilide (CYC) and/or Promalin (PR) applications on feather formation in 'Lapins' / Mazzard sweet cherry trees in the nursery (Expt. 3, 2002) (Quincy, Wash.).

Growth regulator (mg·L ⁻¹) ^z	Leader length (cm)	No./shoot	Induced feathers		Mean length (cm)	Crotch angle (°)
			Union to first induced feather (cm)			
			−PR	+PR		
CYC						
0	209	2.0	131	126	36	39
50	193	4.1	108	117	34	40
100	194	5.7	109	112	32	38
Significance						
CYC linear	*	****	****	NS	NS	NS
CYC quadratic	NS	NS	**	NS	NS	NS
PR						
0	196 a ^y	3.9 a	116 a	---	33 a	38 a
250	202 a	4.0 a	---	118 a	35 a	39 a
Interaction						
CYC × PR	NS	NS	*		NS	NS

²Dilute applications with hydraulic sprayer 5 July 2002 (central leader about 90 cm in length).

³Mean separation in columns by F test ($P \leq 0.05$).

NS,*,**,***,****Nonsignificant or significant analysis of regression at $P \leq 0.05$, 0.01, 0.001, or 0.0001.

Table 5. Effects of cyclanilide (CYC) and/or Promalin (PR) applications on feather formation in 'Bing' / Mazzard sweet cherry trees in the nursery (Expt. 4, 2003) (Quincy, Wash.).

Growth regulator (mg·L ⁻¹) ^z	Trunk caliper (mm)	Leader length (cm)	No./shoot	Induced feathers		
				Union to first induced feather		
				(cm)	Mean length (cm)	Crotch angle (°)
CYC						
0	20 a ^y	242 a	2.7 b	94 a	57 a	41 a
100	19 b	233 b	8.1 a	81 b	45 b	39 b
PR						
0	19 a	240 a	3.7 b	89 a	56 a	40 a
500	19 a	235 b	7.1 a	86 a	46 b	40 a

²Dilute applications with hydraulic sprayer 16 June 2003 (central leader about 70 cm in length).

³Mean separation in columns within treatments by F test ($P \leq 0.05$). No significant CYC × PR interactive effects.

Table 6. Effects of cyclanilide (CYC) and/or Promalin (PR) applications on feather formation in ‘Skeena’/Mazzard sweet cherry trees in the nursery (Expt. 4, 2003) (Quincy, Wash.).

Growth regulator (mg·L ⁻¹) ^z	Trunk caliper (mm)	Leader length (cm)	No./shoot	Induced feathers			Treatment (mg·L ⁻¹)	Mean feather length (cm)
				Union to first induced feather (cm)	Crotch angle (°)			
CYC								
0	24 a ^y	177 a	2.4 b	78 a	36 a		Control	82 a
100	22 a	178 a	7.6 a	72 b	39 a		PR 500	60 b
							CYC 100	43 c
							CYC 100 + PR 500	40 c ^x
PR								
0	23 a	178 a	4.3 b	72 a	38 a			
500	23 a	178 a	5.6 a	77 a	36 a			

^zDilute applications with hydraulic sprayer 16 June 2003 (central leader about 60 cm in length).

^yMean separation in columns by Waller-Duncan Bayesian k ratio test following significant F test for interaction ($P \leq 0.05$).

^xMean separation in columns within treatments by F test ($P \leq 0.05$). CYC × PR interactive effect significant only for mean feather length.

Fig. 2. Experiment 4 (‘Skeena’/Mazzard, nursery, 2003). Note meter stick in each picture. (a) Control trees. Note limited numbers and poor distribution of spontaneous feathers; (b) CYC 100 mg·L⁻¹ applied 16 June; good lateral distribution and development; note cluster of shoots indicating limited action of a single CYC application in reducing apical dominance; (c) PR 500 mg·L⁻¹ applied 16 June; limited feathering; (d) tank-mix of CYC and PR applied 16 June; feathering similar to b.

CYC with PR produced no synergistic effect on feather formation in any trial. CYC appears particularly promising for the production of feathered sweet cherry trees in the nursery because of its strong and consistent stimulation of well-developed lateral shoots. Few other bioregulators have been reported to produce suitable feathering in sweet cherry. Likewise, chemical pinching agents that have been found to induce branching by damaging apple nursery-tree shoot tips have generally shown little effect on sweet cherries (Cody et al., 1985b; Larsen, 1979; Plich and Basak, 1978).

CYC displays an additional advantage for feathering of nursery sweet cherry trees because it does not produce horticulturally significant long-term reduction of or deformation of growth of the terminal meristem even though it temporarily interrupts apical dominance. The development of an equivalent central leader in height after treatment is a critical feature of any branch-induction product for use in the nursery, where a strong central axis carrying several well-developed lateral shoots is the desired tree structure. In these trials, the occasional small effect of CYC on central leader length would not be considered important. The data presented here suggest that CYC has considerable potential as a feathering agent for sweet cherry, especially under nursery conditions.

nursery, the numbers, distribution and growth of these shoots make them unsuitable for re-

trees in the orchard did not result in better feathering than a single application. Combining

Table 7. Effects of cyclanilide (CYC) and/or Promalin (PR) applications on feather formation in ‘Lapins’/Mazzard sweet cherry trees in the nursery (Expt. 5, 2003) (Quincy, Wash.).

Growth regulator (mg·L ⁻¹) ^z	Trunk caliper (mm)	Leader length (cm)	Induced feathers				
			No. -PR	No. +PR	Union to first feather induced (cm)	Mean length (cm)	Crotch angle (°)
CYC							
0	22	273	0.2	4.3	119	53	41
50	22	269	4.1	5.6	114	52	40
100	21	263	5.1	7.5	107	50	38
Significance							
CYC linear	*	*	****	*	***	NS	*
CYC quadratic	NS	NS	**	NS	NS	NS	NS
PR							
0	22 a ^y	266 a	3.1b	---	111 a	51 a	39 a
500	22 a	271 a	---	5.8 a	115 a	52 a	40 a
Interaction							
CYC × PR	NS	NS	*		NS	NS	NS

^zDilute applications with hydraulic sprayer 16 June 2003 (central leader about 95 cm in length).

^yMean separation in columns by F test ($P \leq 0.05$).

NS, *, **, ***, **** Nonsignificant or significant analysis of regression at $P \leq 0.05$, 0.01, 0.001, or 0.0001.

Fig. 3. Experiment 5 ('Lapins'/Mazzard, nursery, 2003). Note meter stick in each picture. (a) Very little spontaneous feather development in control trees; (b) good feathering induced higher up on leaders by CYC 100 mg·L⁻¹ applied 16 June; (c) some feathering induced by PR 500 mg·L⁻¹ applied 16 June; (d) tank-mix of CYC and PR applied 16 June.

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