

Blackheart Injury in ‘Golden Delicious’, ‘Jonagold’, ‘Empire’ and ‘Rome Beauty’ Apple Trees on Five Rootstocks in the 1990 NC-140 Cultivar/Rootstock Trial¹

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Abstract

Blackheart injury was evaluated at 25 cm above the soil surface on ‘Golden Delicious’, ‘Jonagold’, ‘Empire’, and ‘Rome Beauty’ apple (*Malus X domestica* Borkh) trees on M.9 EMLA, B.9, Mark, O.3 and M.26 EMLA rootstocks in the 1990 NC-140 cultivar by rootstock plantings located in Indiana, Iowa, Kentucky, Maine, Massachusetts, Ohio, and Tennessee. Trees grown in Iowa, which recorded the coldest temperatures, exhibited the greatest injury, while trees grown in Tennessee, Massachusetts, and Maine exhibited the least injury. Cultivar susceptibility to blackheart injury was affected by location, with Jonagold trees sustaining a high percentage of injury in Iowa and Indiana, and Rome trees sustaining high injury in Indiana, Kentucky, Maine, and Massachusetts. Trees on Mark rootstock, followed by B.9 and M.9 EMLA, most frequently exhibited a high percentage of blackheart injury, while trees on M.26 EMLA, followed by O.3, most frequently sustained less blackheart injury. Supplemental decline ratings of trees in the Iowa planting in conjunction with the blackheart injury support reports that trees on Mark and B.9 rootstocks lack cold hardiness in mid to late winter when exposed to fluctuating temperatures.

Low temperature tolerance of apple trees on dwarfing rootstocks is a concern in colder apple-producing regions. Trees have been killed following test winters, but more often exhibit symptoms of decline from which recovery often occurs. Injury can be to the roots or the above-ground portions of the trees. Root injury typically occurs under conditions where trees are growing on sandy soils and absence of snow cover during a freezing event (16). Depending upon when the freezing event occurs, injury to the above-ground portions of the trees can be to the xylem, bark, or buds. In mid-winter, apple vegetative buds and bark are much hardier than living xylem cells, while xylem is hardier than the bark and buds in early autumn and late spring (17, 18). Blackheart is a form of winter injury characterized by the killing of xylem parenchyma cells and the occlusion of vessel elements (22). The typical symptom of blackheart is oxidative browning of the xylem tissue (20) caused by the supercooled fraction of intercellular water freezing in the xylem ray parenchyma cells (17, 18). Most often, blackheart injury does not cause outright death of the plants with recurring injury being common in nature, and it has been implicated in the decline and reduced productivity of fruit trees (17).

Several reports have attempted to rank rootstock cold tolerance based upon root hardiness (4, 6, 7, 16), while others have attempted predict above-ground hardiness based on controlled freezing of

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one- or 2-year-old rootstock shoots (4, 6, 9, 10, 12). Studies evaluating the influence of rootstocks on scion hardiness are limited, and results have been mixed with some showing rootstock differences (7, 19, 23, 24, 25) and others reported no differences (8, 21). Previous multi-site uniform apple rootstock and interstem plantings established by NC-140 have served as excellent means for studying the influence of rootstocks on the winter hardiness of scion cultivars through the evaluation of blackheart injury (19, 23, 24). In 1990, multi-state uniform apple rootstock study was initiated at 17 sites to evaluate the performance of four cultivars representing different growth habits on five rootstocks found to be most promising in previous NC-140 plantings (14). The purpose of this study was to assess the relative susceptibility of ‘Golden Delicious’, ‘Jonagold’, ‘Empire’, and ‘Rome Beauty’ to blackheart injury on five rootstocks in the NC-140 cooperative apple plantings located in Indiana, Iowa, Kentucky, Maine, Massachusetts, Ohio, and Tennessee.

Materials & Methods

Apple plantings were established in 1990 and maintained at West Lafayette, Indiana; Ames, Iowa; Princeton, Kentucky; Monmouth, Maine; Belchertown, Massachusetts; Wooster, Ohio; and Crossville, Tennessee according to guidelines established by the NC-140 committee (14). ‘Smoothie Golden Delicious’, ‘Nicobel Jonagold’, ‘Empire’, and ‘Law Rome Beauty’ were propagated on M.9 EMLA, B.9, Mark, O.3, or M.26 EMLA rootstocks, and planted at a spacing of 3 x 5.5 m in a randomized-complete-block/split-split plot design with 6 single-tree replicates as described by Autio et.al (1)

In November 1999, blackheart injury was recorded for all remaining trees at each location. Trunks were cross sectioned at 25 cm above the soil surface with a chain saw, and the percentage of blackheart injury was determined from tracings of the total xylem and blackheart xylem (24). The percentage of blackheart was calculated from cutouts of the tracings either on an area basis with the aid of a leaf area meter (LI-COR, Lincoln, NE), or on a weight basis. An alternative approach to tracing was photocopying trunk sections to obtain total xylem and blackheart xylem cutouts.

Data collection and analyses were organized by the Massachusetts site cooperator. Analyses of variance were conducted with the MIXED procedure of the SAS software package (SAS Institute, Cary, NC). To calculate the overall cultivar, rootstock, and cultivar-by- rootstock least-squares means, cultivar, rootstock and the interaction of cultivar and rootstock were considered fixed effects, while location, replication nested within location and all additional interactions were considered random, and analyzed as described by Autio et.al (1). To calculate location, and main effects and interactions within location, cultivar, rootstock, location, and the interactions among these main effects were considered fixed effects, and analyzed as described by Autio et.al (2).

Results & Discussion

Low-temperature events were recorded at each location during the 10-year period of the study (Table 1). Iowa experienced the coldest conditions with temperatures $\leq -30^{\circ}\text{C}$ during three winters. Maine (incomplete records), follow by Indiana and Ohio, were the next coldest locations. Massachusetts, Kentucky and Tennessee experienced the mildest winters. Some of the coldest

temperatures recorded across locations were in January 1994, and February 1996. Tree loss from winter injury was minimal in this study, and was confined to three trees of 'Jonagold'/Mark in the Iowa planting. With the exception of the loss of plantings in Minnesota and Montana in the 1980-81 NC-140 trial due to severe winter injury (13), low tree mortalities from winter injury have been reported in previous NC-140 rootstock studies (19, 23, 24).

The percentage blackheart data was first analyzed with cultivar, rootstock, and the cultivar-by-rootstock interaction as fixed effects, and location, replication within location and all other interactions considered random. Over all rootstocks, 'Rome' trees sustained greater blackheart injury than 'Golden Delicious' trees (Table 2). Over all cultivars, rootstocks had no effect on blackheart injury. However, the cultivar-by-rootstock interaction was significant and when repartitioned as the effect of rootstock within cultivar, differences within 'Rome' were evident, with trees on Mark exhibiting greater blackheart injury than trees on B.9, M.26 EMLA, and O.3 (Table 2).

Blackheart injury was affected significantly by location, and interactions with location were evident. Overall, trees in the Iowa planting, which were exposed to the coldest temperatures, sustained the greatest injury, while trees in Tennessee, Massachusetts, and Maine were the least injured (Table 3). Trees in the Indiana, Kentucky, and Ohio plantings exhibited intermediate injury. As in previous studies, blackheart injury was not confined to the coldest locations (23, 24). In addition, the severity of blackheart injury between locations was not directly related to the minimum temperatures recorded at the sites. This was particularly evident for Maine which was the second coldest location (Table 1) and experienced some of the least blackheart injury. The severity of injury between locations appeared to be more closely related to the potential for fluctuating winter temperatures (continental verses maritime climatic conditions) in association with minimum temperatures recorded.

Location and cultivar interacted significantly to affect injury, and the data were repartitioned to evaluate cultivar within location (Table 3). In Iowa, 'Jonagold' trees sustained the greatest injury, while in Indiana, 'Jonagold' and 'Rome' exhibited greater blackheart injury than 'Golden Delicious' or 'Empire'. In Kentucky, Maine, and Massachusetts, 'Rome' trees exhibited the greatest blackheart injury, but were not different from 'Empire' or 'Jonagold' in Maine, or 'Jonagold' in Massachusetts. No difference between cultivars was found in Ohio or Tennessee. No difference between 'Empire' and 'Golden Delicious' was found at any location, or between 'Empire' and 'Jonagold' at five of the locations. Also, 'Rome' sustained greater injury than 'Golden Delicious' at four of the locations. Based upon controlled freezing of one-year-old shoots, Forsline (10) reported that 'Golden Delicious' and 'Jonagold' exhibited a low tolerance to freezing throughout the winter, while 'Empire' was generally as hardy as 'McIntosh'. In late winter controlled freezing of 2-year-old wood, 'Empire' was least sensitive, and 'Jonagold' was most sensitive to freezing (12). Forsline (10) also reported that 'Rome' was as hardy or hardier than 'McIntosh' in December, but did not continue further testing. Based upon field observations, 'Rome' has been reported to be less hardy than 'McIntosh' but hardier than 'Baldwin' (11). Our results suggest that controlled freezing of young tissue is not a reliable indicator of whole-plant low-temperature tolerance under field conditions.

Location and rootstock interacted significantly to affect injury, and the data were repartitioned to evaluate rootstock within location (Table 3). In Iowa, trees on Mark exhibited the greatest blackheart injury, while in Indiana, trees on Mark had more blackheart than trees on M.26 EMLA. In Massachusetts, trees on M.9 EMLA exhibited greater blackheart than trees on B.9, Mark, or M.26

EMLA. Rootstocks did not significantly affect blackheart injury in Kentucky, Maine, Ohio, or Tennessee. However, in Indiana, Iowa, Kentucky, Maine, and Massachusetts cultivar and rootstock interacted significantly to affect blackheart injury, and the data were repartitioned by location to analyze the effect of rootstock within each cultivar (Table 4). Where significant differences existed among rootstocks, no consistent pattern was evident within a cultivar among locations or within a location among cultivars, however, trends were evident. Generally, trees on Mark were most frequently associated with a high incidence of blackheart injury (80%). Where Mark had a high incidence of injury, trees on B.9 and M.9 EMLA were not different from Mark 63% and 57% of the time, respectively. Trees on M.26 EMLA, followed by trees on O.3, most frequently sustained significantly less injury than Mark (63% and 57% of the time, respectively). Only in the Kentucky planting did 'Empire' trees on O.3 sustain greater injury than trees on Mark.

The Iowa planting was exposed to several freezes (-22°C on 7 Nov. 1991; -28°C on 24 Feb. 1993 following a thaw; -32°C on 19 and 30 Jan. 1994; -36°C on 2 Feb. 1996 following a mid-January thaw; and -23°C on 12 Mar. 1998 following a thaw) that affected tree vigor. Beginning in 1992, the trees were rated annually in August on a scale of 1 to 6 for symptoms of decline (5). Differences between cultivars were evident following the February freezes in 1993 and 1996 (Table 5). 'Empire' trees exhibited greater symptoms of decline than 'Golden Delicious' trees in 1993, while in 1996, 'Empire' exhibited greater symptoms than 'Golden Delicious' or 'Rome'. When compared to cultivar influence on blackheart injury (Table 3), there appeared to be no relationship between the two evaluations. However, the decline ratings probably were confounded by differences in cultivar growth habit which ranged from the spur-type and basitonic 'Empire' to the tip-bearing and acrotonic 'Rome'. Trees exhibited signs of recovery following winters without a severe freeze.

A rootstock effect on tree vigor was evident in 1992 following the November 1991 freeze, with trees on Mark exhibiting the greater decline symptoms than those on M.9 EMLA and M.26 EMLA (Table 5). Following the Feb. 1993, Feb. 1996, and Mar. 1998 freezes, trees on Mark exhibited the greatest decline symptoms, and least recovery in years following the freezes. In 1993, trees on B.9 exhibited greater decline symptoms than trees on M.26 EMLA and O.3, and in 1996, trees on B.9 exhibited greater decline symptoms than trees on M.9 EMLA, M.26 EMLA, or O.3. Recovery was evident in years following non-stressful winters, with no differences detected between trees on M.9 EMLA, B.9, O.3, and M.26 EMLA. Significant cultivar-by-rootstock interactions were found following the Feb. 1996 and Mar. 1998 freezes, and the data were repartitioned and analyzed as rootstock within cultivar. For 'Golden Delicious' in 1996, B.9 was not different from M.9 EMLA, O.3, or M.26 EMLA (data not shown). For 'Empire' in 1998, there was no difference between Mark and B.9 (data not shown). Generally there appeared to be a close relationship between the rootstock effect on visual ratings and the percentage blackheart injury in the Iowa planting (Table 3). Lack of vegetative growth has been associated with blackheart injury (5, 14). Steinmetz (20) reported that damage may not be apparent when less than 20% of the xylem was injured, while trees sustaining greater than 50% injury may not recover. Based upon the Iowa tree-vigor ratings and blackheart injury, it is probable that apple trees can sustain greater than 50% xylem injury and recover.

In the 1980-81 NC-140 rootstock trial, trees on MAC.9 (Mark) exhibited a high incidence of blackheart injury in the Iowa and Quebec plantings (23). Although Mark has been reported to acclimate early and tolerate below average seasonal temperatures(9), it has been reported susceptible

to injury in mid and late winter (5, 25). The tree-vigor ratings obtained on the Iowa planting following three mid to late winter freezing events in conjunction with the high percentage of blackheart injury sustained by trees on Mark provides further evidence that Mark is not cold tolerant in mid- to late-winter. Our results also support other reports that M.26 EMLA and O.3 exhibit high level of cold tolerance (5, 6, 12, 24).

Forsline (10) reported that M.9 was relatively non-hardy throughout the winter. In the 1980-81 NC-140 rootstock trial, no difference in blackheart injury was found between trees on M.9, M.9 EMLA, and M.26 EMLA (23). In this study, only for the Massachusetts and Kentucky plantings within 'Rome' did trees on M.9 EMLA exhibited a significantly greater percentage of blackheart injury than trees on another rootstock (Table 4).

B.9 has generally been considered tolerant to mid-winter freezes on an order similar to M.26 (3), but often it has been categorized based on root hardiness (4, 16). In our study, trees on B.9 exhibited a high incidence of injury where significant location-by-rootstock interactions existed (Table 4). 'Starkspur Supreme Delicious' trees on B.9 sustained a high incidence of blackheart injury in the 1984 NC-140 rootstock trial (24). Embree (8) reported a high percentage of blackheart following a February freeze when B.9 was used as a framebuilder. In a study where several rootstocks were exposed to above freezing temperatures for up to 10 days in January, February, and March, B.9 deacclimated more rapidly than M.9, M.26, P.2 or P.22 (4). The Iowa tree-decline ratings following exposure to -28°C on 24 Feb. 1993 after a thaw and -36°C on 2 Feb. 1996 after a thaw (Table 5), in conjunction with the blackheart injury sustained by 'Jonagold' and 'Empire' trees in the planting (Table 4), are further evidence that trees on B.9 lack cold hardiness in mid to late winter when exposed to fluctuating temperatures. Since temperature conditions in Indiana would be most similar to those experienced in Iowa, the differences in blackheart injury sustained among rootstocks within 'Jonagold' and 'Rome' in that planting would provide additional support.

In conclusion, multi-site uniform apple rootstock plantings established by NC-140 continue to serve as an excellent means for studying the influence of rootstocks on the winter hardiness of scion cultivars through the evaluation of blackheart injury. These studies serve to expose rootstocks to a wide array of climatic conditions and have effectively assessed the influence of rootstock on scion hardiness on a short period of time without experiencing a test winter.

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Table 1. Minimum monthly temperatures (°C) at seven locations from November 1990 through March 1999.^z

Month	Year	IN	IA	KY	ME	MA	OH	TN
November	1990	-	-14	-4	-7	-6	-6	-11
December	1990	-	-27	-12	-14	-12	-16	-17
January	1991	-19	-28	-12	-24	-20	-19	-14
February	1991	-17	-20	-13	-22	-15	-16	-23
March	1991	-7	-8	-4	-	-7	-9	-8
November	1991	-14	-22	3	-10	-6	-12	-12
December	1991	-17	-20	-9	-20	-15	-13	-11
January	1992	-23	-23	-15	-23	-19	-20	-18
February	1992	-12	-12	-12	-24	-21	-14	-12
March	1992	-11	-13	-9	-23	-13	-15	-9
November	1992	-4	-14	-5	-14	-7	-6	-8
December	1992	-14	-20	-12	-23	-14	-12	-8
January	1993	-16	-23	-11	-26	-17	-12	-10
February	1993	-22	-28	-16	-32	-22	-19	-16
March	1993	-11	-21	-10	-19	-18	-15	-15
November	1993	-8	-15	-8	-14	-12	-6	-8
December	1993	-17	-23	-12	-23	-21	-19	-14
January	1994	-30	-32	-27	-30	-24	-31	-26
February	1994	-20	-26	-12	-24	-21	-20	-13
March	1994	-7	-11	-7	-17	-17	-9	-7
November	1994	-4	-9	-4	-	-8	-15	-9
December	1994	-7	-20	-8	-	-13	-10	-8
January	1995	-18	-23	-14	-	-16	-17	-18
February	1995	-19	-21	-15	-	-24	-20	-20
March	1995	-12	-23	-6	-	-11	-13	-8
November	1995	-11	-17	1	-13	-8	-8	-10
December	1995	-19	-23	1	-22	-17	-17	-18
January	1996	-24	-31	-17	-25	-23	-21	-16
February	1996	-27	-36	-17	-25	-23	-22	-26
March	1996	-17	-21	-13	-15	-19	-15	-18
November	1996	-13	-17	-7	-12	-8	-11	-7
December	1996	-18	-23	-16	-21	-19	-13	-15
January	1997	-12	-26	-20	-25	-21	-20	-18
February	1997	-4	-24	-5	-24	-12	-14	-7
March	1997	-1	-13	-7	-17	-11	-9	-8
November	1997	-1	-10	-11	-11	-9	-9	-11
December	1997	-3	-20	-7	-19	-17	-13	-12
January	1998	-13	-22	-7	-	-17	-12	-16
February	1998	-7	-14	-6	-	-15	-7	-4
March	1998	-17	-23	-13	-	-15	-10	-12
November	1998	-7	-5	-3	-	-5	-3	-7
December	1998	-19	-23	-11	-	-17	-11	-13
January	1999	-29	-32	-13	-	-21	-16	-17
February	1999	-11	-9	-8	-	-15	-9	-10
March	1999	-14	-8	-4	-	-12	-11	-6

^z Trees were planted in spring 1990. IN = Indiana, IA = Iowa, KY = Kentucky, ME = Maine, MA = Massachusetts, OH = Ohio, TN = Tennessee.

Table 2. Blackheart injury as affected by cultivar and rootstock after 10 years in the 1990 NC-140 Cultivar/Rootstock Trial. All values are least-squares means, adjusted for missing subclasses. Cultivar and rootstock interacted significantly to affect injury, so mean separations are presented for rootstock within each cultivar.^z

Rootstock	Golden Delicious	Jonagold	Empire	Rome	Mean
<i>Blackheart injury (% trunk cross-sectional area affected)</i>					
M.9 EMLA	15 a	25 a	17 a	35 ab	23 a
B.9	17 a	26 a	19 a	23 c	21 a
Mark	21 a	26 a	18 a	37 a	26 a
O.3	15 a	26 a	16 a	25 bc	21 a
M.26 EMLA	15 a	22 a	19 a	24 c	20 a
Mean	17 b	25 ab	18 ab	29 a	

^zMean separation among rootstocks or among cultivars by t test ($P = 0.05$) with a Bonferroni adjustment (adjusted $P = 0.005$ for rootstocks, adjusted $P = 0.008$ for cultivars).

Table 3. Blackheart injury (% of trunk cross-sectional area damaged) as affected by cultivar, rootstock, and location in the 1990 NC-140 Cultivar/Rootstock Trial. All values are least-squares means, adjusted for missing subclasses.^z

Cultivar/Rootstock	Indiana	Iowa	Kentucky	Maine	Massachusetts	Ohio	Tennessee
M.9 EMLA	25 ab	43 b	21 a	---	22 a	21 a	10 a
B.9	28 ab	50 b	17 a	13 a	9 b	20 a	8 a
Mark	33 a	61 a	19 a	19 a	10 b	22 a	14 a
O.3	25 ab	43 b	36 a	11 a	15 ab	17 a	6 a
M.26 EMLA	23 b	44 b	24 a	13 a	10 b	15 a	9 a
Golden Delicious	19 b	43 b	19 b	7 b	6 b	18 a	6 a
Jonagold	41 a	65 a	13 b	14 ab	12 ab	18 a	8 a
Empire	16 b	38 b	16 b	16 ab	10 b	17 a	9 a
Rome	32 a	45 b	39 a	22 a	23 a	23 a	13 a
Mean ^y	27 b	48 a	23 bc	15 cd	13 cd	19 bc	10 d

^zMean separation among rootstocks or among cultivar by t test ($P = 0.05$) with a Bonferroni adjustment (adjusted $P = 0.005$ for rootstocks, adjusted $P = 0.008$ for cultivars).

^yMean separation by Tukey's HSD ($P=0.05$).

Table 4. Blackheart injury (% of trunk cross-sectional area damaged) as affected by cultivar, rootstock, and location in the 1990 NC-140 Cultivar/Rootstock Trial. All values are least-squares means, adjusted for missing subclasses. In Indiana, Iowa, Kentucky, Maine, and Massachusetts, cultivar and rootstock interacted significantly to affect injury, so mean separations are presented for rootstock within each cultivar.^z

Cultivar	Rootstock	Indiana	Iowa	Kentucky	Maine	Massachusetts
Golden Delicious	M.9 EMLA	---	33 b	---	---	10 a
	B.9	19 a	42 b	21 a	---	6 a
	Mark	21 a	60 a	16 a	9 a	7 a
	O.3	19 a	40 b	---	6 a	6 a
	M.26 EMLA	18 a	39 b	17 a	8 a	4 a
Jonagold	M.9 EMLA	42 ab	56 b	---	---	17 a
	B.9	31 ab	73 a	---	14 a	12 a
	Mark	46 a	74 a	2 a	17 a	7 a
	O.3	51 a	63 ab	15 a	---	---
	M.26 EMLA	30 b	61 ab	19 a	12 a	11 a
Empire	M.9 EMLA	20 a	35 ab	13 bc	---	11 a
	B.9	18 a	44 ab	17 bc	16 a	8 a
	Mark	19 a	49 a	5 c	20 a	4 a
	O.3	6 a	31 b	50 a	14 a	13 a
	M.26 EMLA	19 a	34 ab	26 ab	13 a	15 a
Rome	M.9 EMLA	27 b	46 ab	49 ab	---	47 a
	B.9	39 ab	40 b	21 c	16 ab	10 b
	Mark	45 a	59 a	64 a	32 a	20 b
	O.3	31 ab	39 b	---	7 b	25 b
	M.26 EMLA	23 b	42 b	34 bc	20 ab	11 b

^zMean separation among rootstocks within cultivar by t test ($P = 0.05$) with a Bonferroni adjustment (adjusted $P = 0.005$).

Table 5. Annual tree vigor ratings as affected by cultivar and rootstock in the Iowa planting of the 1990 NC-140 Cultivar/Rootstock Trial. All values are least-squares means, adjusted for missing subclasses.^Z

Cultivar/Rootstock	1992 ^Y	1993	1994	1995	1996	1997	1998	1999
Golden Delicious	1.4 a	1.8 b	1.3 a	1.1 a	1.6 b	1.3 a	1.3 a	1.0 a
Jonagold	1.6 a	2.1 ab	1.6 a	1.4 a	1.9 ab	1.5 a	1.5 a	1.5 a
Empire	1.4 a	2.3 a	1.6 a	1.3 a	2.2 a	1.2 a	1.7 a	1.1 a
Rome	1.3 a	2.1 ab	1.1 a	1.2 a	1.7 b	1.4 a	1.3 a	1.2 a
M.9 EMLA	1.2 b	1.9 bc	1.0 b	1.0 b	1.3 c	1.1 b	1.0 b	1.0 b
B.9	1.7 ab	2.3 b	1.5 b	1.2 b	2.2 b	1.2 b	1.5 b	1.2 b
Mark	1.8 a	2.8 a	2.3 a	2.0 a	3.5 a	2.4 a	2.7 a	1.8 a
O.3	1.3 ab	1.7 cd	1.2 b	1.0 b	1.2 c	1.0 b	1.0 b	1.0 b
M.26 EMLA	1.2 b	1.5 d	1.0 b	1.0 b	1.1 c	1.0 b	1.0 b	1.0 b

^ZMean separation by Tukey's HSD ($P=0.05$).

^YRating scale 1 (healthy) to 6 (dead).