METHYL BROMIDE ALTERNATIVES, FOCUS ON ROOTSTOCKS

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ABSTRACT

Our walnut rootstock studies began in 1997 with initiation of the Paradox Diversity Study. Our first three years were occupied with evaluation of the nematode host status of 3500 individual root systems in search for possible resistance among paradox seedlings or their associated black walnut parent. During those studies we identified: 1) a black walnut called AW269 (Rawlins seedling) that might have resistance to root lesion nematode. 2) 4 of 10 seedlings of NX and 3 of 10 seedlings of UZ that exhibited some type of pre-infection resistance mechanism that protected their roots the first year they were in the ground. 3) A single paradox referred to as VX211 that exhibited high vigor even in the presence of nematode feeding. While Wes Hackett and Chuck Leslie were propagating for us the above listed rootstocks, we initiated studies of three English clones with Janine Hasey (published 2005) and then three more clones with Bob Beede. Root systems of *Juglans* are already 99% protected from nematodes, presumably due to phenols, tannins and antioxidants within the more developed root parts. In conducting studies with Bob Beede we were amazed to find resistance in Serr to *Meloidogyne incognita* nematodes. In a 1984 evaluation that included Serr we had not seen such resistance so we obtained 18 more own-rooted Serr from Burchell Nursery plus Serr grafted onto a Northern California Black. One-third of the NCB stocks supported *M. incognita,* some at high populations, but not a single *M. incognita* has been found after searching a total of 28 own-rooted Serr trees for two years. It is still unclear how supportive Serr clones are for *Pratylenchus vulnus* but they are certainly not resistant to this nematode. There will be more data on Serr in 2008. Importantly, we are stratifying 380 seeds of the Afghanistan parent of Serr and those trees will be screened for nematode development in 2009. In addition, we began in fall 2005 our search for nematode resistance among more elite sources of *Juglans* spp. We can now report the first example of a *Juglans* spp with apparent resistance to *M. incognita* and *P. vulnus*. More specifically, we have found that 5 of 11 selections of *J. cathayensis* do not host either of these nematodes. In this annual report we will focus on our progress relative to nematode resistance mechanisms identified during the last decade of study.

OBJECTIVES

1) Maintain the Rio Oso trial for yield data, rates of nematode return and tree growth.
2) Remove up to half the trees (6 reps) from the VX211 and AX1 trial at Kearney Ag Center and graft over to Chandler for yield data.
3) Obtain for further nematode and field evaluations the clones of RX032 and UZ229 (Wes Hackett).
4) Continue nematode evaluations of various *Juglans* species from the USDA Davis Repository.
5) Compare all *Juglans* selections in adjacent replicated fumigated and non-fumigated sites in two walnut replant orchards, one following NCB the other Paradox.
6) Quantify the number of root tips/tree, their length, and the length of each root tip that will host *P. vulnus* and *Meloidogyne* spp.

7) Explore at our KAC trial site the value of Garlon + one year of chemical drenches and/or non-hosts as methods for reducing soil populations of *P. vulnus*, *Mesocriconema xenoplax* and *Meloidogyne* spp. down to various soil depths.

PROCEDURES

Objective 1. The Rio Oso site is now sixth leaf and involves approximately 8 acres with every other tree being either NX or DN paradox. These rows have been planted over soil that received either methyl bromide to the 6-foot depth, Telone II at 50 gallons per acre stripped with delivery to the 4.5-foot depth or a broadcast application of Metam sodium delivered to the 4-foot depth.

Objective 2. Within 48 macroplots (1/100th acre each) at KAC we have 12 reps each of VX211 and AX1 in each without nematodes plus 36 reps with nematodes. In fall 2006 we cut the trunks within 1/3 of these macroplots and applied Garlon. The remaining 4 or 8 reps will be grafted to Chandler for purposes of yield collection. Nematode sampling of the soil and trunk measurements will continue twice yearly.

Objective 3. Selection RX032 is a *Juglans microcarpa* that we selected because it performed well in our earliest nematode screens. A second *J. microcarpa* was also saved but its vigor level after 7 years is half that of RX032 so it is being terminated. UZ229 is of interest because it is now the largest of three saved UZ trees. These three trees were originally selected because three out of ten appeared to keep *P. vulnus* away from their roots, very similar to what was observed with the NX seedlings. Wood will be submitted to Wes Hackett again this year for further nematode evaluations. Approximately 13 UZ229 were received from Dr. Hackett last year and they are currently receiving evaluation against *P. vulnus* on a lath house bench. RX032 is of interest for its host status against nematodes but also for its growth habit against the rejection component.

Objective 4. In winter 2005-06 we received small amounts of *Juglans* seed from Ed Stover of the Germplasm Repository. These were planted into nematode inoculated soil at KAC. The most vigorous trees today include *J. hindsii* and *J. californica.* In addition we planted varying numbers of *J. regia, J. microcarpa, J. major, J. ailantifolia, J. mandshirica, J. cathayensis* and *J. nigra.* The *J. nigra* trees did not survive and more *J. microcarpa* are needed. We also received from Tom Burchell own-rooted Serr trees and Serr grafted onto NCB. These trees were all inoculated with *P. vulnus* and *Meloidogyne* spp. on June 15, 2006 and thus far the only trees that have been removed for examination are one each of Serr own- rooted and Serr on NCB. In 2007 and beyond the Serr trees will be completely removed by backhoe to examine all roots but the other trees will be kept alive and only a portion of their roots removed every six months for two years.

Objective 5. Any trees worthy of future evaluation plus all the trees received from the repository will be sized for uniformity and then planted into adjacent fumigated and non-fumigated sites within a walnut replant site for two years to determine size differences. We usually require six trees for each rep and reps are needed because the intensity of the rejection component varies across a field. It will be difficult to find the rejection component without the presence of *P. vulnus*. Each year we will compare visible differences in growth as well as trunk diameters to determine if trees grow similar whether fumigated or not. We refer to this measurement as tolerance to the rejection component.

Objective 6. By carefully removing entire first-year trees with a backhoe we are able to capture most of the root system. We dissect the root system into: 1) root initials, their number and length, 2) fibrous roots that support the tips <7 mm in diameter and 3) roots larger than 7 mm diameter. Walnut roots whether English, Black or the hybrid tend not to support more than three *P. vulnus*/gram of root. Our interest is in the number of initials, their size and the portion of the root that does not support high population levels. Plant data are tabulated and each column analyzed statistically. Nematode counts are collected from each of these root groupings and also analyzed for differences.

Objective 7. Milo and true sudan grass are non hosts of *P. vulnus* in field settings. Many other plants are as well but following a year of milo, for example, it is not unusual to find very few *P. vulnus* in the surface 2 feet of soil. This is the reason we recommend soil samples to 3-feet deep when searching for *P. vulnus* if the orchard has been out for a while. Acrolein is a weed killer that is used in aquatic settings such as ditches, lakes, ponds. It is also a good nematicide when applied to the 5-foot depth at 250 ppm in 6 inches of water. Further, NCB grows very well in the years following such applications.

We halted studies on acrolein back in 1994 because at 250 ppm the aldehyde odor was unbearable and adjacent barley was damaged. When applied at 7to14 ppm to waterways, none of these odor problems arise. I want to apply acrolein as a drench while milo or sudan grass are present and absent to determine how deep we can deliver nematicidal activity. Additionally, we now know that the walnut hull extract called NatureCur is quite nematicidal. Unlike most biological derived products NatureCur follows a dosage response curve in that if you apply more you get more kill. We want to use it as a pre-plant treatment with and without sudan grass or milo. Each of these chemical products should receive registration without much problem. These plants and products and perhaps others will receive evaluation in our old VX211 sites at KAC where we now have *P. vulnus* to five feet and the soil is perfect for drenching. If successful nematode control is achieved we will replant with a variety of walnut selections by spring 2008.

Treatments for each of these objectives will be replicated at least four times but our limitation will likely be the availability of adequate walnut selections. All nematode counts will be log-transformed and subjected to ANOVA.

RESULTS

The value of pre-infection resistance

In 2001 we planted half the trees in an 8-acre orchard near Rio Oso, CA using NX paradox seedlings. The other half was planted with a local favorite, DN paradox seedlings. These two rootstocks were installed as a sub plot overlaying a main plot that involved three reps each of three different fumigation treatments. Fumigation treatments included: a) MB broadcast that was delivered 6 foot deep, b)Telone/vapam on 10 foot wide strips that delivered protection to 4.5 foot depth, and c) Vapam (MS) drenched broadcast to 4-feet depth. These treatments provided us with varying levels of nematode return, but more importantly the actual volume of nematode-free soil varied considerably because of these differing soil fumigations. From the early years of this trial we could observe from trunk measurements that NX seedlings possessed greater uniformity from tree to tree compared to the DN seedlings. In fall 2007 this difference has become even more noticeable along with the slightly greater vigor associated with NX seedlings. The 2007 trunk diameters presented in Table 1 indicate the value of fumigating large volumes of soil (MB broadcast) versus small volumes of soil (Telone stripped). Table 1b indicates the degree of nematode protection provided by the pre-infection resistance of NX. These seedlings are unavailable for propagation so in 2004 we requested Wes Hackett provide us with clones of UZ229, a mother tree that after 7 years had gradually reached almost the same size as VX211. We received clones of UZ229 in spring 2006 along with several other rootstocks of interest including: Vlach, VX211, RX1, PX1, AX1, UZ229, WIP 3, RX032 and AZ205. Five of these were grown in a lath house for a full year in the presence of *P. vulnus* and *M. incognita* nematodes and the nematode counts from each selection are presented in Table 2. Once again, what we had termed pre-infection resistance (poor nematode buildup in the first year) has apparently provided us with a rootstock that supports fewer nematodes at its root tips than those provided by all other selections currently available. This is not a display of resistance, but is a display of an active resistance mechanism. We believe this mechanism will be most valuable if the trees are planted where nematodes are not very abundant at the time of planting. In spring 2007 we transferred these lath house grown trees infected with nematodes into a field setting with an adjacent row of the same clones one year younger and free of nematodes. In late fall of 2007 we will re-sample these trees but at the time of this writing we have indicated their first-year heights in Table 2b. Relative to NX and UZ229 our hypothesis is that something leaking out of the roots of some NX and UZ selections is directly or indirectly reducing nematode attack and that this is manifest as reduced nematode reproduction.

The value of a selection exhibiting superior vigor

Compared to all other paradox selections the superior growth of VX211 was noticeable 6 months after planting. Even at two full years after planting in the presence of nematodes the growth of this selection stood out above all other VX selections and all other paradox selections. We wanted to understand if the extreme growth advantage of this seedling was due to its superior vigor or to its tolerance because it supported an abundance of *P. vulnus.* To answer this question we obtained from Wes Hackett 48 clones of VX211 and 48 clones of a standard paradox seedling, AX1. These two clones were planted adjacent to each other in 1/100 acre macroplots where soil had been infested with 0, 1, 20 or 500 *P. vulnus* nematodes per 250 cc of soil. The superior vigor of VX211 was apparent four months after planting. Tree growth was significantly reduced among both clones by the presence of even a single nematode / 250 cc of soil. The term ‘tolerance’ was difficult to accept in year 2 because VX211 and AX1 were responding quite similar to nematode presence except that at low inoculum levels the nematode counts were diminishing from VX211 compared to AX1. These separations in nematode buildup and tree growth were not apparent until year 3 but they have continued into the fourth leaf. In summer 2007 a colleague versed in root staining was hired to quantify nematode presence within the root tips of VX211 compared to AX1. The root tips of VX211 do not support nematodes within their tissues but do support their feeding and reproduction along the outside surface of the roots. VX211 carries a resistance mechanism that excludes nematode entry deep into root tips, particularly root tips that are longer than 3 cm in length (see Table 3). Meanwhile, AX1 does not have this mechanism and generally permits nematode feeding outside and inside the terminus 15 cm of its roots. Feeding that is limited to the root epidermis provides a mechanism that is relatively unique to VX211 and is likely responsible for any tolerance that VX211 exhibits. In summary, VX211 is highly vigorous, appears to tolerate nematode feeding and carries resistance within its root tips but not at their epidermis. The lack of endoparasitic feeding associated with VX211 is different from the pre-infection resistance of UZ and NX. Of the 63 trees grafted in 2007 to Chandler scion, our grafting success was 93%, and 79% for VX211 and AX1, respectively. A publication on our VX211 studies is in progress.

Resistance to root knot but not root lesion

When we began these studies the dogma was that Northern California Black (NCB) was resistant to *M. incognita* but English was not and Paradox was somewhere in between. There may be some general truth to this assessment but each seedling selection appears to differ a bit. We now have good resistance to *M. incognita* in own-rooted Serr compared to NCB seedlings. For the Rawlins NCB 1/3 of our seedlings were a good host for *M. incognita* while the other 2/3 were not. These data are presented in Table 4. There is also a confounding factor when conducting studies with mixed species of nematodes around walnut. One may not see the impact of *M. incognita* if it is inoculated with *P. vulnus*. These two nematode genera compete with one another and it is *P. vulnus* that eventually wins the competition. Resistance in walnut to *P. vulnus* will surely open the door to *M. incognita* attack unless the rootstock also contains resistance to *M. incognita*.

Resistance to root knot and root lesion nematodes

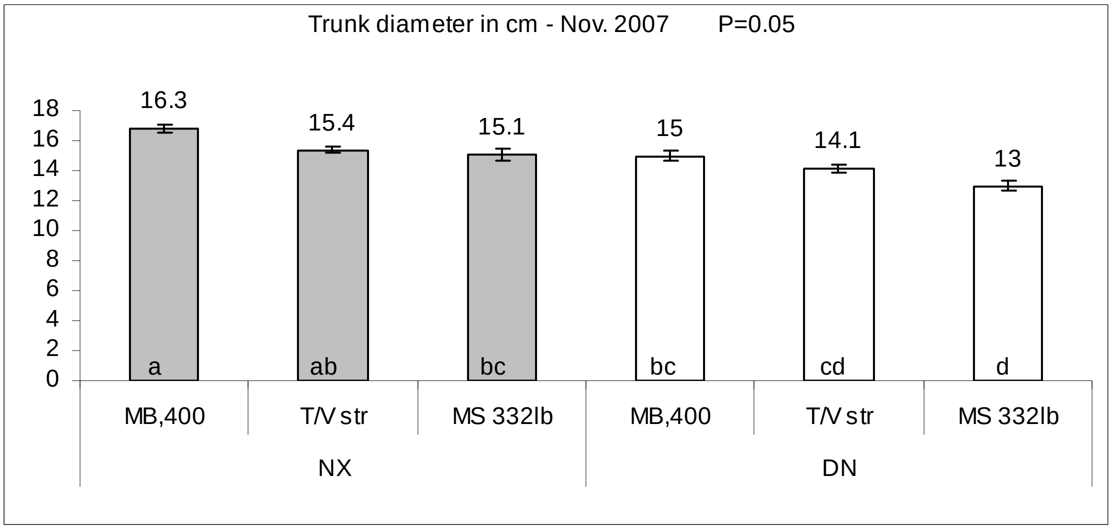
For this researcher the designation of resistant is reserved for plants that support fewer than 0.2 endoparasitic nematodes per gram of root after a two-year study. Our 2005 planting of elite *Juglans* spp has led us to such a plant. We call it *J. cathayensis* #21. After two full years this selection is as vigorous as any of the neighboring *Juglans* trees, including NCB. Five of the 11 seedlings of *J. cathayensis* supported 0.0 nematodes per gram of root, see Table 5. These nematodes, *M. incognita* and *P. vulnus,* were also absent from soil after two years. This finding will prove to be a huge asset in our search for a *Juglans* spp. having very different parentage but also resistance to nematodes.

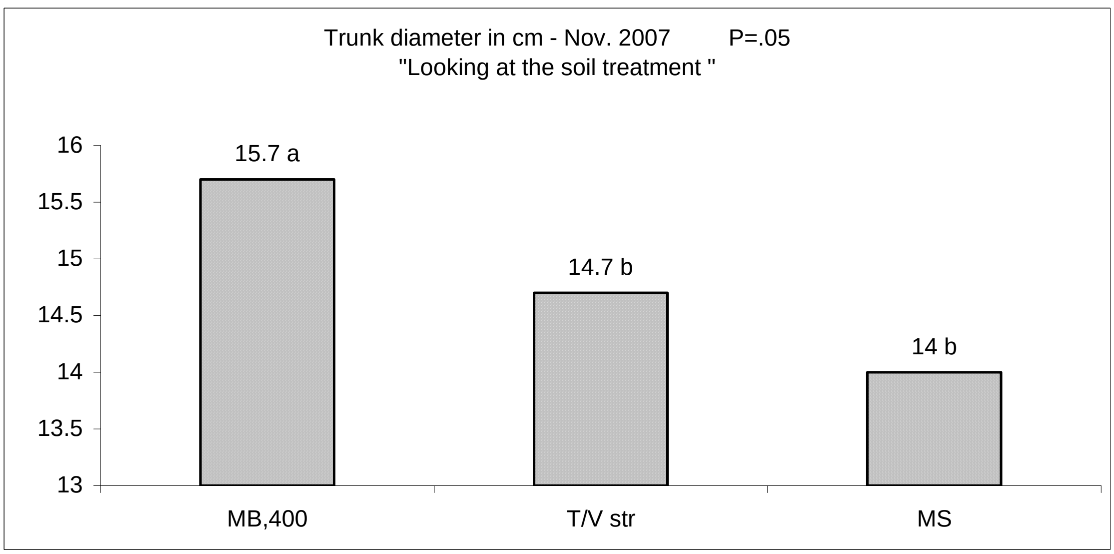
DISCUSSION

A full decade after initiation of our search for nematode resistance in walnut rootstocks, this investigator has the optimism that there are useful sources of resistance among *Juglans* spp. Our steps toward new rootstocks have been small but each step has shown that notable nematode resistance can provide useful tools in the end. In fact, success is almost guaranteed. This is because *Pratylenchus vulnus* builds to such high populations in young walnut roots of contemporary *Juglans* rootstocks. Review the data from Table 2 and observe that during their first year in the ground VX211 supports 1307 *P. vulnus* per gram of root. This is a very large number and yet we find this selection to be an improvement when compared to other Paradox selections that host five times that number. As a reference point we refer to nematode counts in excess of 180/gram of root to be an indication of high susceptibility.

As indicated in earlier reports 99+% of walnut root biomass provides a relatively poor food base for nematodes. The youngest roots, 6 inches of tissue spread across the existing root system, are attractive to *P. vulnus* at <1 nematode / 250 cc soil sample. The result is tremendous population buildup. Extensive population buildup coupled with 5% of the population surviving five years without feeding leads us to the conclusion that any reductions in population development could have important long-term consequences relative to overall root system size. This report quantifies to varying degrees the value of pre-infection resistance mechanisms associated with NX and UZ229. There is also in VX211 some mechanism that relegates nematode feeding to the exterior of the root, thus leaving the internal portions of the roots protected. There is in Serr a mechanism that halts feeding by *M. incognita.* This may be the same mechanism that halts their feeding in many black walnut selections (NCB). Most recently we have identified some astounding resistance mechanism(s) in Chinese Walnut, *J. cathayensis*. Each of these findings has direct value for rootstock breeding programs but some products of our research are already available (VX211 and Serr) or close to being available at the grower level (UZ229).

Table 1. Rio Oso trunk differences at knee height in 2007, 6th leaf walnut.





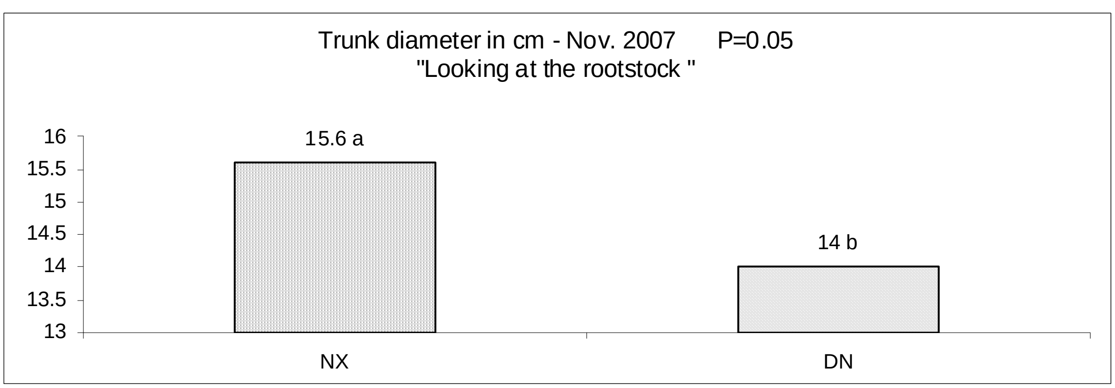


Table 1b. Incidence of *P. vulnus* 6 years after various soil treatments at Rio Oso.

Soil Treatment	*** Volume of soil fumigated / acre**	**Mean *P. vulnus* / 250 cc soil when replanted to NX Paradox ****	**Mean *P. vulnus* / 250 cc soil when replanted to DN Paradox ****
400 lb/acre MB (tarp) broadcast	99.99% control in 24 M lb soil/acre	2 a	123 ab
500 lb/acre rate Telone stripped + 110 lb/acre metam sodium	99.99% control in 9 M lb soil/acre	301 bc	145 abc
325 lb/acre metam sodium broadcast in 6 acre-in water	99.90% control in 16 M lb soil	432 c	332 c

* Data from each foot to 5 feet deep 60 dat.
** Data from top 2 feet at edge of tree canopy.

Table 2. First-year development of five Paradox selections against *P. vulnus* in a lath house experiment.

Rootstock	**Nematodes/Gram of Root**
Vlach	6580 a
PX1	1944 b
AX1	1426 bc
VX211	1307 c
UZ229	730 c

Note: we refer to nematode resistance as <0.2 nematodes/gram of root. Nematode counts in excess of 180/gram of root we refer to as highly susceptible.

Table 2b. First-year tree height of nine Paradox clones with and without *P. vulnus*.

Rootstock	**Height without Pv**	**Height with Pv**	**ANOVA at *P* = 0.05**
RX032	136 cm	132 cm	ns
RX1	119 cm	95 cm	*
VX211	117 cm	128 cm	ns
AZ025	110 cm	80 cm	ns
AX1	92 cm	86 cm	ns
Vlach	84 cm	56 cm	*
WIP 3	80 cm	37 cm	*
PX1	75 cm	86 cm	ns
UZ229	62 cm	82 cm	ns

Note: Rootstocks with significant height differences are indicated by an asterisk.

Table 3. Preference of *P. vulnus*, nematodes/gram of washed root, at three positions along the root terminus of two paradox clones one-year after planting.

Clone	**root tips (0 to 3cm distal)**	**3.1 to 9cm distal**	**9.1 to 15 cm distal**	**associated 250cc soil**
VX211	5.9 a	7.9 a	4.0 a	881 a
AX1	271. b	345 b	103 b	541 a

Means within each column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at *P* = 0.05.

Table 4. Preference of *P. vulnus* and *M. incognita* / gram of terminus root for English 'Serr' versus Northern California Black 'Rawlins' roots at 18 mo after nematode inoculation.

	Serr	**Rawlins**
P. vulnus / gram root terminal 0 – 15 cm roots	526 a	125. b
M. incognita / gram root terminal 0 – 15 cm roots	0.0 a	259. b

Note: The fleshy terminus roots of NCB are typically 10 to 15 cm in length compared to 3 to 10 cm lengths from roots of English stocks. Means along each row followed by a similar letter are not significantly different according to Duncan's Multiple Range Test at P = 0.05.

Table 5. Two-year development of *Pratylenchus vulnus* and *Meloidogyne incognita* on 11 individual trees of *Juglans cathayensis* (Jc) and *Juglans ailantifolia* (Ja).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**mean**
roots												
Jc Pv/gr	0	5.3	0	25	1.8	45.8	465	0	0	0	0.1	49.3 a
Ja Pv/gr	259	319	453	25	728	615	2	885	1507	–	–	533 b
Jc Mi/gr	0	0	0	0.7	0	0	0	0	0	0	5.6	0.6 ns
Ja Mi/gr	0	0	0	54	0	0	64.8	0	0	–	–	13.2
soil												
Jc Pv/250cc	0	145	0	484	8	22	1108	0	0	0	0	161 a
Ja Pv/250cc	426	624	1960	0	2576	2009	17	1768	1672	–	–	1228 b
Jc Mi/250cc	0	0	0	0	0	0	0	0	0	0	45	4.1 ns
Ja Mi/250cc	19	0	0	55	0	0	644	0	0	–	–	79.8

Means followed by a different letter are significantly different (P = 0.05).