A6.

Ranking of HLB Research Priorities

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The rapid global expansion and growing economic damage caused by HLB has prompted citrus industries worldwide to explore investing in research that has potential to provide tools for mitigation/control of the epidemics before these industries succumb to the disease. Various research priorities have been identified in previous HLB meetings and workshops. Recently, both the National Academy of Sciences (NAS) and USDA ARS have compiled lists of current and future research areas that can be used by universities, federal and state government agencies, and citrus industries to prioritize and fund research. These areas mostly fall under the following broad general categories.

Economics	Alternate Hosts
Detection of Disease/Vector	Differentiation
Resistance and Breeding	Culturing HLB
Pathogen/Vector Interactions	Citrus Genetics
Chemical Control	Biological Control
Cultural Control	Pathogenesis
Epidemiology	Transgenics
Genomics	Vector Biology
Fruit Yield/Quality	

Delegates of the conference were asked to examine potential research priorities that were identified both by NAS and ARS expert panels. To accomplish this, survey forms were distributed during the IRCHLB and delegates were asked to rank the research priorities listed. Forms were created for this purpose and are shown below (Figures 1 and 2). Conference delegates were asked to indicate their affiliation in categories of: Researcher/Scientist, Citrus Industry, or Regulatory. Delegates were then asked to rank each of the research priorities on the basis of: A) Probability of Success, B.) Impact if Successful, and C.) Duration, i.e., Short versus Long Term, each of these was ranked on a 1 to 5 (low to high) scale as indicated on the survey forms.

Survey data were summarized in two ways. First as an overall ranking by adding the ranking scores of the three criteria A) Probability of Success, B.) Impact if Successful, and C.) Duration. Results of this first ranking are shown in Figures 3 to 6 for the NAS-identified priorities, and in Figures 7 to 10 for the ARS-identified priorities, in descending order. Second, the rankings were broken into the three criteria and are displayed in 3-D graphs, with each of the criteria on a separate axis, for both the NAS (Figures 11 to 13) and ARS (Figures 14 to 18) research priorities, respectively. For Figures 11 to 18, each of the individual ranked priorities is identified by its respective ID number on the 3-D graphs.

The 3-D graphs demonstrate that the dispersion of the ranked priorities was moderately clustered by Research/Scientist (Figures 11, 12, 14) and Regulatory delegates (Figures 15, 16, 18), whereas, Citrus Industry delegates tended to rank the priorities in a more dispersed fashion (Figures 13 and 17).

Acknowledgment: *We thank K. Poole, G. Poole, and A. Ford for data input during the conference*.

Figure 1. HLB Research Priorities Survey Form - Page 1.

Page 1.

International Research Conference on Huanglongbing (IRCHLB)
Orlando, FL December 1-5, 2008
Research Priorities

Affliliation

☐ Researcher/Scientist **☐ Citrus Industry** **☐ Regulatory**

NAS Identified Research Priorities 2008	For each Research Priority, rank the probabliity of success, impact and duration from 1 to 5, with 1 being the lowest (shortest) and 5 being the highest (longest). **A)** Probability of Success (1 - 5)	**B)** Impact if Successful (1 - 5)	**C)** Duration-- Short vs Long term (1 - 5)
1. Perform Koch's postulates to conclusively demonstrate that Liberibacter is the causative agent of HLB and investigate biological properties of any relevant co-cultures.			
2. Identify psyllid repellents from guava volatiles and other sources.			
3. Construct Liberibacter genomic DNA library. Sequence and assemble full-length genome sequence.			
4. Determine if modified spray techniques/applications etc. can improve psyllid control.			
5. Develop transformation systems for mature tissue of commercial varieties (both rootstocks and scions).			
6. Determine if RNA interference can be used to manipulate psyllid gene expression.			
7. Develop improved (faster and more sensitive) assays for Liberibacter using PCR-, antibody-based, remote sensing, or other methods of detection.			
8. Identify proteins or peptides that have anti-Liberibacter activity.			
9. Examine the effects of oils and particle films on psyllids and HLB transmission.			
10. Characterize the microbiome of citrus phloem tissue.			
11. Identify attractants (chemical, color) in psyllid hosts and from other sources.			
12. Examine effectiveness of flush management in controlling citrus psyllid interactions and HLB transmission. Ways to improve flush management include high-density planting using micrografting and tree dwarfing (with dwarfing factors, e.g. different rootstock).			
13. Save Rutaceous germplasm; Screen citrus and Vepris germplasm for Liberibacter resistance and initiate citrus breeding to develop HLB resistant citrus.			
14. Identify biological proteins (Bt-like) that affect the psyllid.			
15. Examine the relationship between environmental factors (light intensity, temperature, etc.) and HLB symptoms and timing of host and pathogen responses.			
16. Develop HLB resistant citrus by mutagenesis and selection.			
17. Perform detailed Liberibacter transmission studies using psyllid, Liberibacter and citrus genotypes found in Florida.			
18. Determine if Liberibacter is seed transmitted in citrus.			
19. Develop a more facile (non-citrus) model system for HLB.			
20. Identify factors related to variability of psyllid acquisition of pathogen; establish why less than 100% of psyllids will acquire Liberibacter.			
21. Develop fast assay for detecting pathogen in the psyllid.			
22. Determine the latency period between inoculation of citrus with Liberibacter and development of HLB symptoms, under different pathogen titers and different host conditions (phenology).			
23. Study effects of anti-microbial compounds on HLB in citrus.			
24. Examine transcript, protein and metabolite levels in Liberibacter infected citrus to better understand the plant's response to HLB.			
25. Determine if citrus explants in tissue culture support high levels of Liberibacter.			
26. Grow nursery and production stock under screen until fruit-bearing age.			
27. Conduct economic feasibility studies of alternative citrus production systems (i.e. high density planting, hydroponics, screen house propagation/culture, intercropping with guava).			
28. Develop a detailed understanding of the mechanism of HLB transmission, including Liberibacter acquisition time, mechanism of attachment to psyllid, inoculation period. Determine the mechanism and location of attachment of Liberibacter in psyllids.			
29. Examine life cycle of psyllid in detail, including mating cues (acoustics), migration/dispersal patterns, stylus formation, etc.			
30. Develop better methods for monitoring and determining the presence of psyllids in the environment.			
31. Develop psyllids by breeding or transgenic approaches that do not vector Liberibacter.			
32. Synthesize repellents or engineer microbes to synthesize repellants.			
33. Sequence an EST library of psyllid genes.			
34. Examine seasonal distribution of Liberibacter in citrus (both levels and location within a tree). Analyze the distribution of Liberibacter in symptomatic and asymptomatic citrus tissues.			
35. Develop molecular research tools for Liberibacter including monoclonal antibodies to Liberibacter, reference strains of Liberibacter and psyllids, cDNA and genomic libraries of Liberibacter.			
36. Examine effectiveness of different methods to stimulate systemic acquired resistance to HLB in citrus.			

Page 2.

ARS Researchable Areas from ARS HLB Workshop: 4-23-08

CROP IMPROVEMENT	**A)** Probability of Success (1 - 5)	**B)** Impact if Successful (1 - 5)	**C)** Duration-- Short vs Long term (1 - 5)
Plant host resistance to HLB			
1. Transgenic/cisgenic resistant varieties and rootstocks.			
2. Induced resistance w/ SAR.			
3. Virus-based host resistance (to pathogen or vector) for screening/therapy.			
4. Conventional breeding rootstocks.			
5. Host response mechanisms. Direct RNA sequencing (compare healthy vs infected plants) e.g., role of miRNAs in disease development.			
VECTOR BIOLOGY AND MANAGEMENT			
Psyllid biology			
1. Vector biology, seasonality, environmental effects, life history, dispersal.			
2. Vector capacity, survival, transmission efficiency, propensity.			
3. Vector genomics, including expression.			
Psyllid resistance and response to the HLB pathogen			
1. Transgenic psyllid			
2. Endosymbiont targets (e.g., Wolbachia/virus transformation for Liberibacter control)			
3. Strain diversity; host-compatibility groups			
4. Histopathology of psyllid-vector relationship			
Psyllid-based control			
1. Urban areas? Homeowner psyllid management?			
2. Area-wide program?			
3. Symbiont manipulation for vector control			
4. Novel pesticide development (RNAi targeted at specific psyllid genes for treatment of vector, BT library evaluation)			
5. Insecticide resistance management			
6. Insect repellent plant development ("virtual nets")			
7. Mating disruption by…sound/pheromones, etc.			
8. Plant Volatiles (attractants-Murraya, repellents)			
9. Natural enemies, esp. for unmanaged citrus, e,g., parasites, predators, fungal/viral pathogens of insect			
10. Kaolin clay applications			
11. Barrier-based control (plants or netting impregnated with insecticide)			
12. Wind breaks with lures and chemical treatment (trap plant)			
PATHOGEN AND DISEASE BIOLOGY AND DETECTION			
Pathogen biology and detection			
1. Pathogen detection			
2. Genome sequence			
3. Culture organism to supplement current effort (high throughput methods)			
4. Improved diagnostics in psyllid and plant tissue			
5. Pathogen variability and diversity			
6. ID factors critical to pathogenesis, virulence: Functional genomics, gene expression, proteomics, direct RNA sequencing			
7. Population biology and spatial and temporal distribution in planta			
8. Asymptomatic plant host range e.g., source of resistance, reservoir for inoculum			
Disease biology			
1. Etiology (Koch postulates for Liberibacter., metagenomics from phloem and psyllid endophytes)			
2. Sources of variability in symptoms (interaction with other microbes, endophytes, stress interactions)			
3. Early disease detection (non-pathogen based)			
4. High throughput disease detection (e.g., imaging techniques, hyperspectral detection or laser or microwave detection, trained dogs)			
5. Seed transmission and seed treatment			
6. Rescue of infected germplasm/breeding material from field/seed			
EPIDEMIOLOGY AND DISEASE MANAGEMENT			
Epidemiology and Management of HLB			
1. Symptom remission methods (antibiotics, others? Injection methodology)			
2. Intercropping for disease management (e.g., guava)			
3. Volatile effects and identification			
4. Replacement tree protection via physical/chemical barriers (e.g., slow release "virtual net")			
5. Repellents/avoidance factors (e.g., kaolin itself and as a carrier)			
6. Cultural methods (e.g., nutrient delivery, early production systems, flush management, open hydroponic systems)			
7. Effect of climate on disease biology (e.g. effect on range of endemic Liberibacter and psyllid, grow in Northern FL?)			
8. Fruit/juice quality and abscission (productivity and quality in infected and treated trees)			

Figure 3. Ranked NAS Priorities

Figure 4. Ranked NAS Priorities

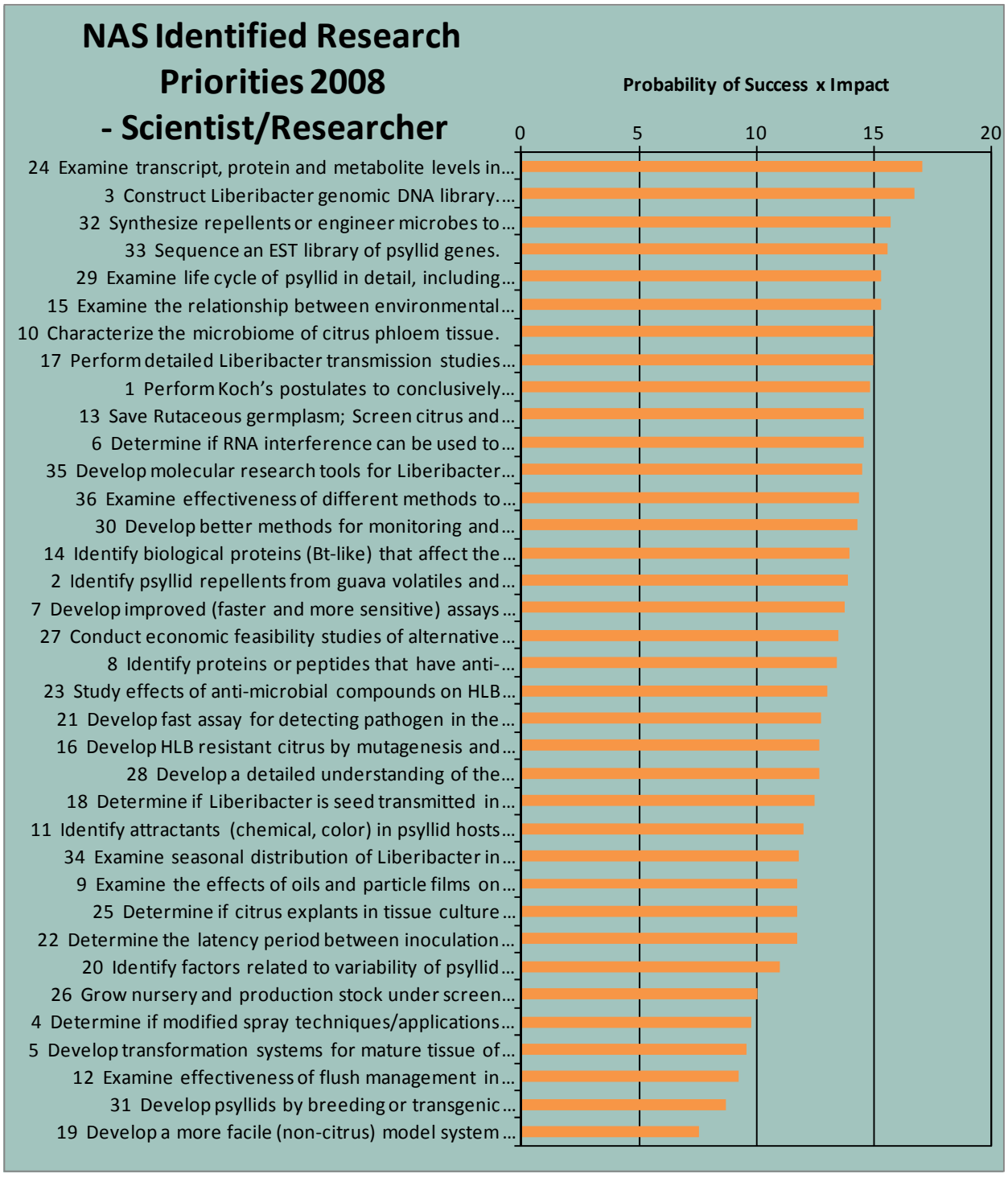

Figure 5. Ranked NAS Priorities

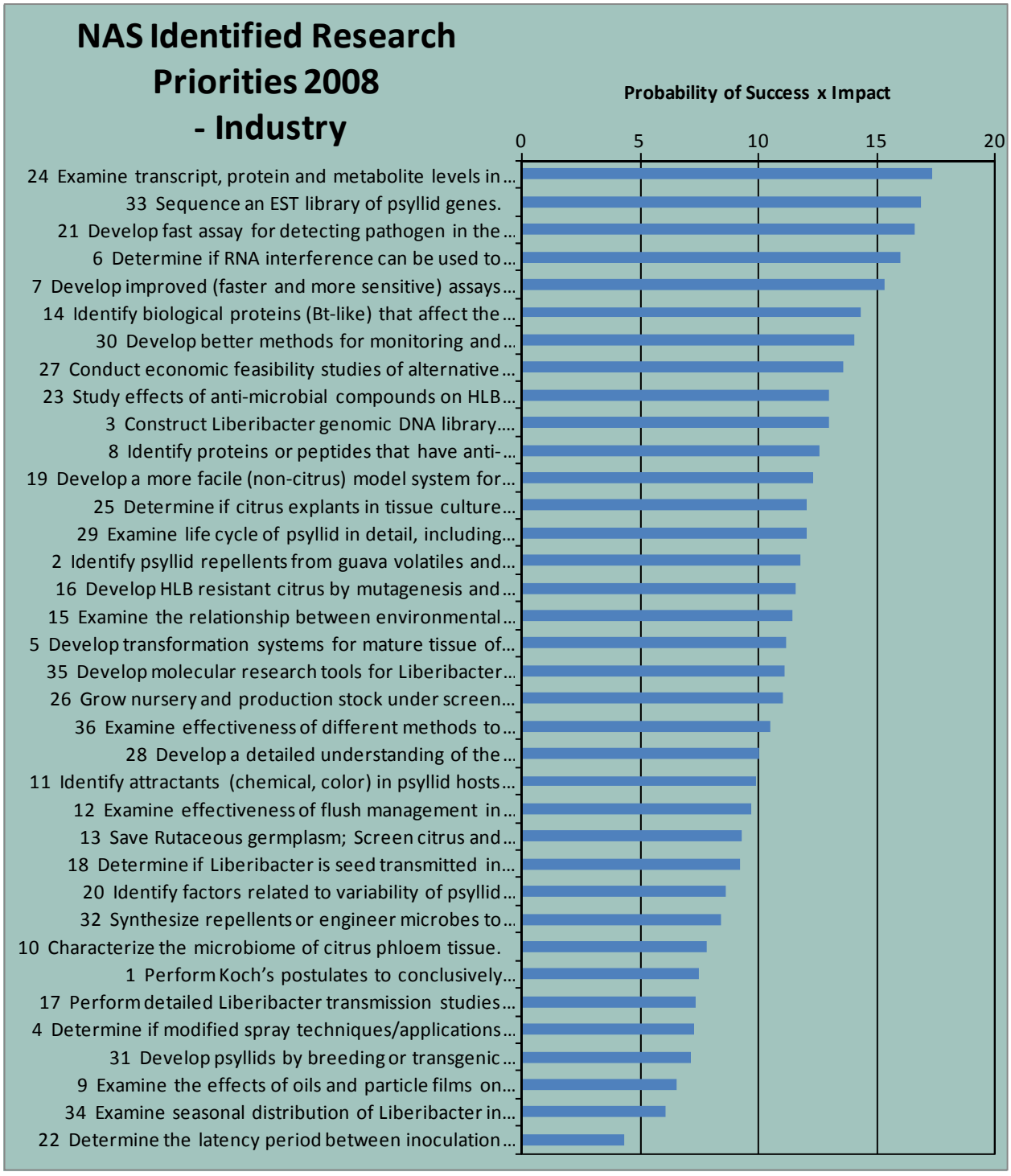

Figure 6. Ranked NAS Priorities

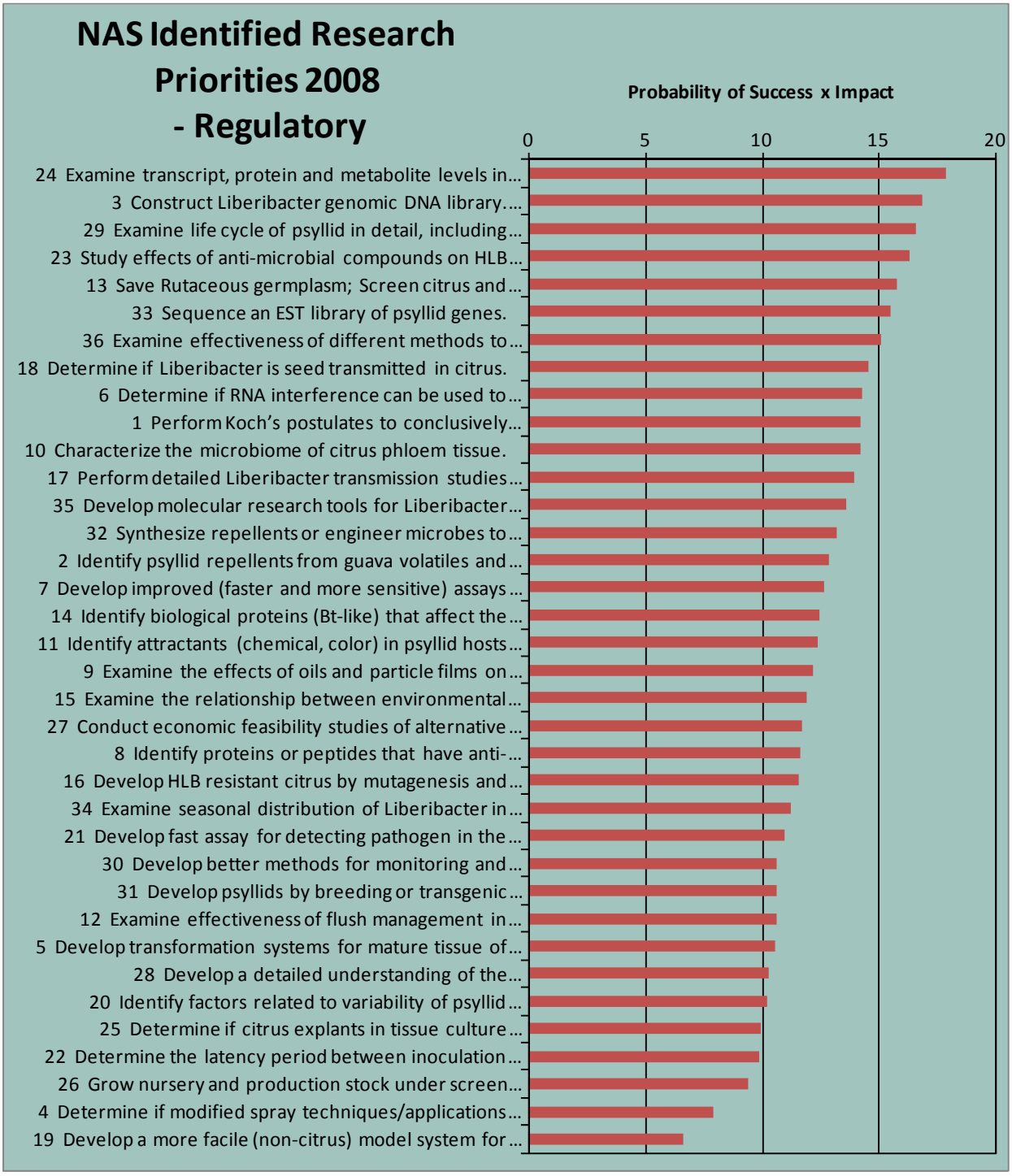

Figure 7. Ranked ARS Priorities

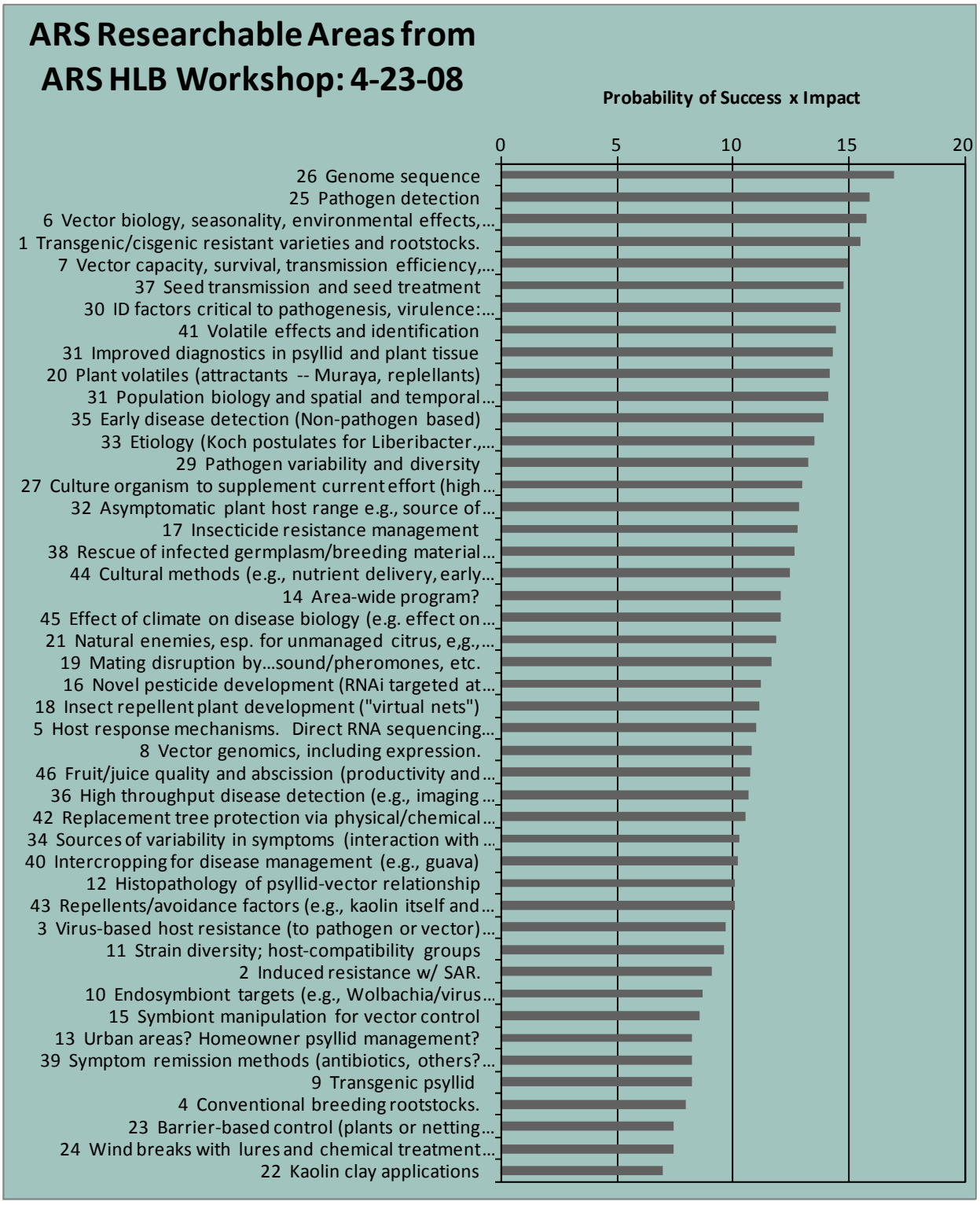

Figure 8. Ranked ARS Priorities

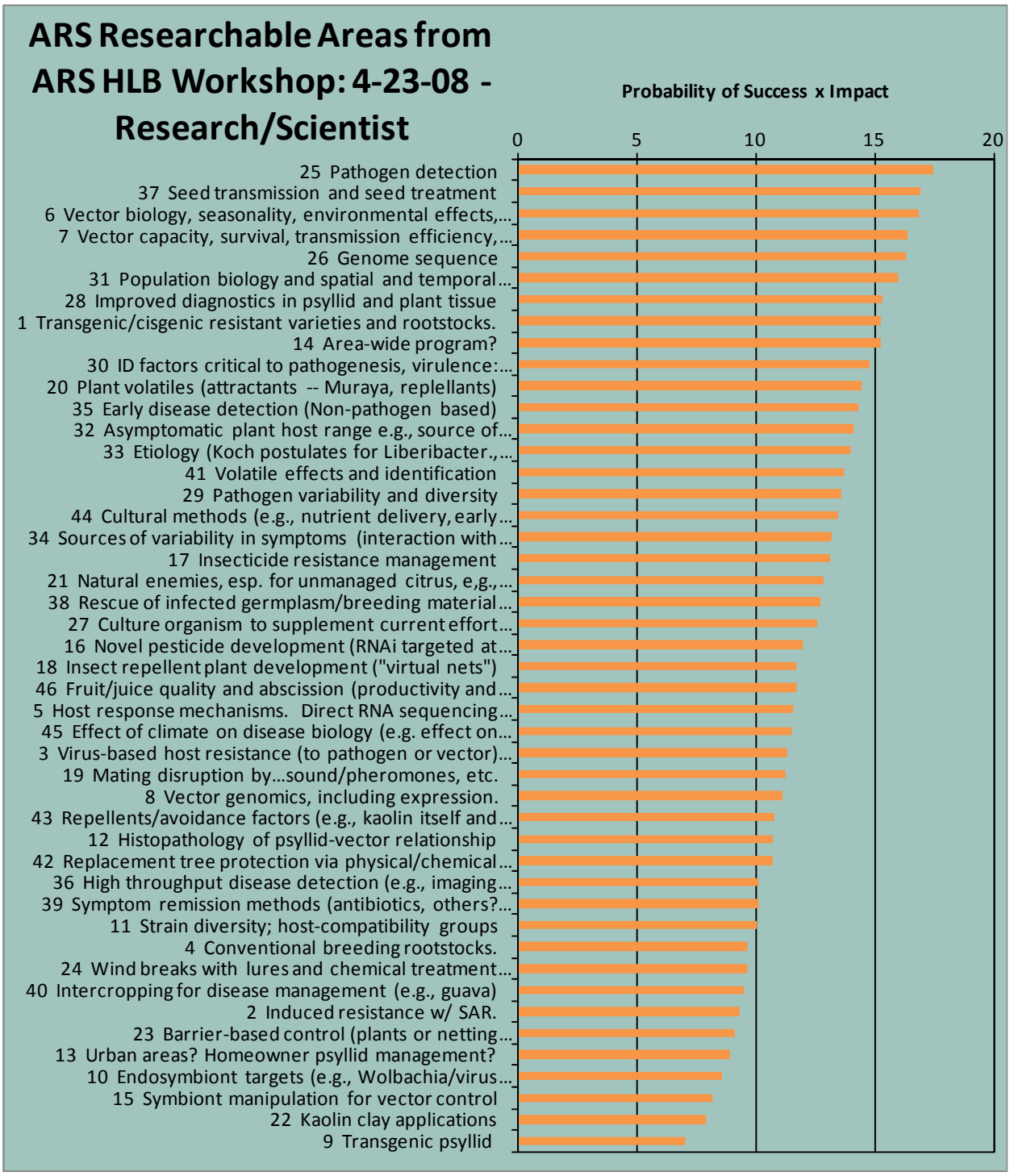

Figure 9. Ranked ARS Priorities

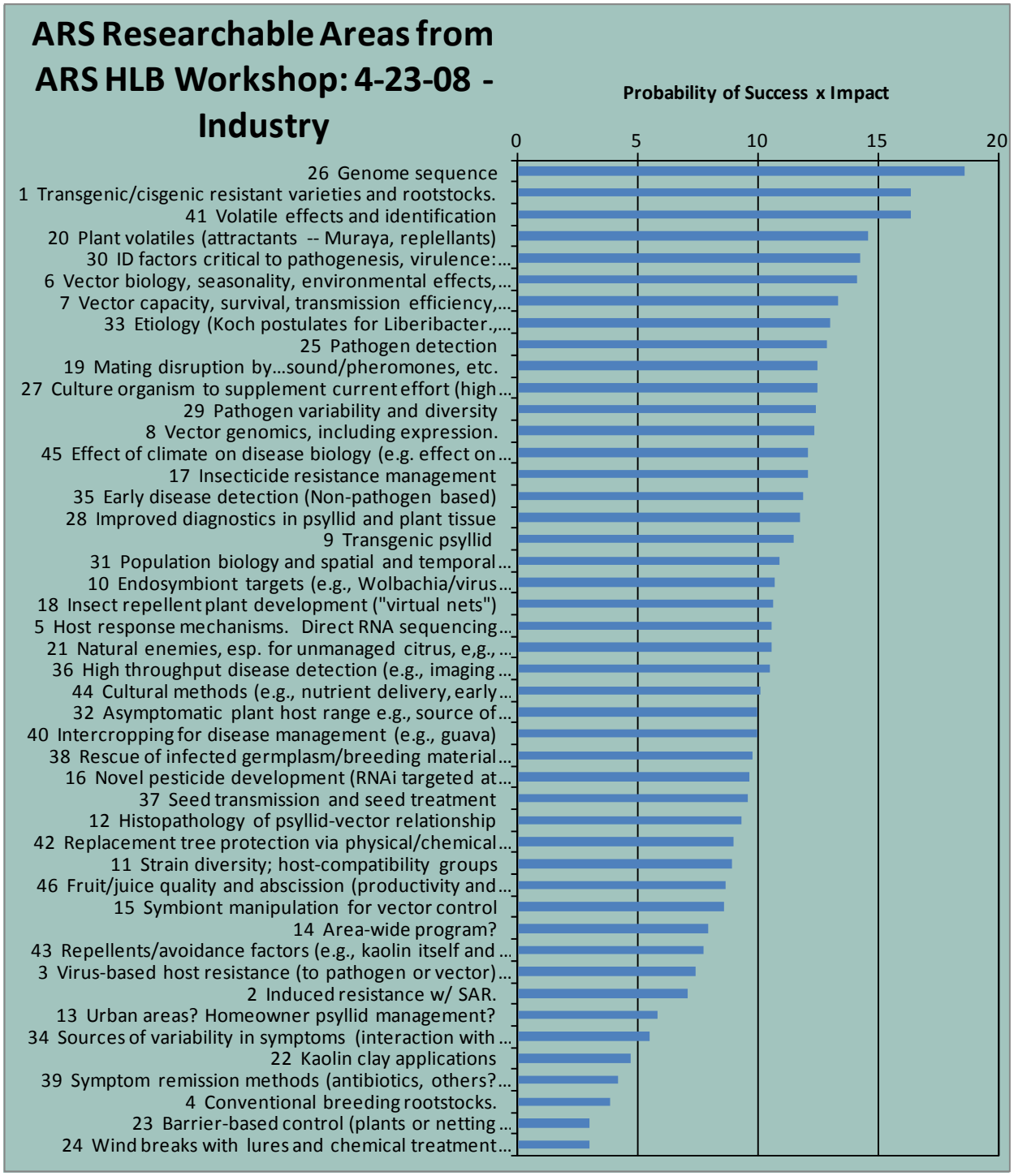

Figure 10. Ranked ARS Priorities

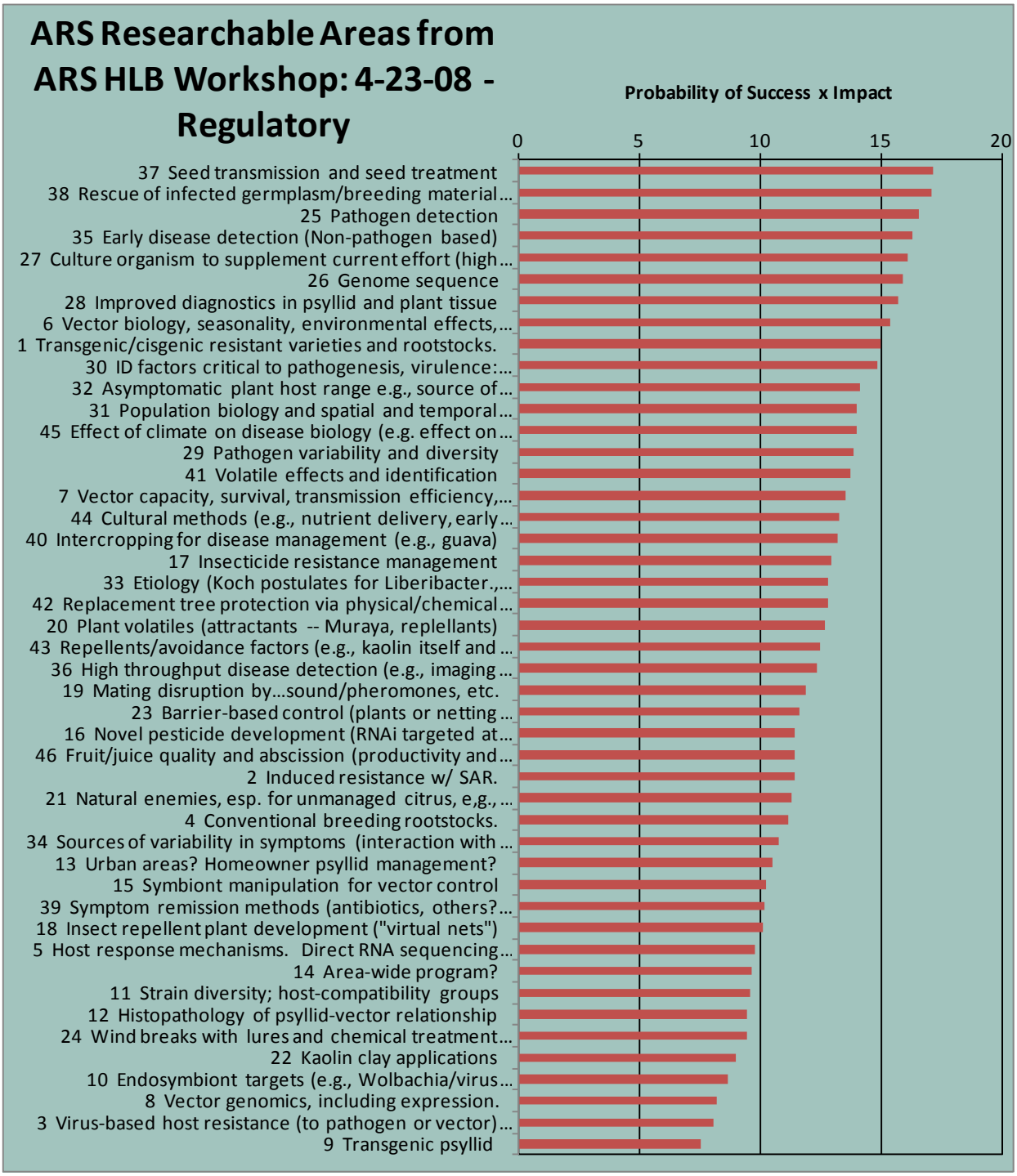

Figure 11. NAS Ranking of Impact vs. Success vs. Duration

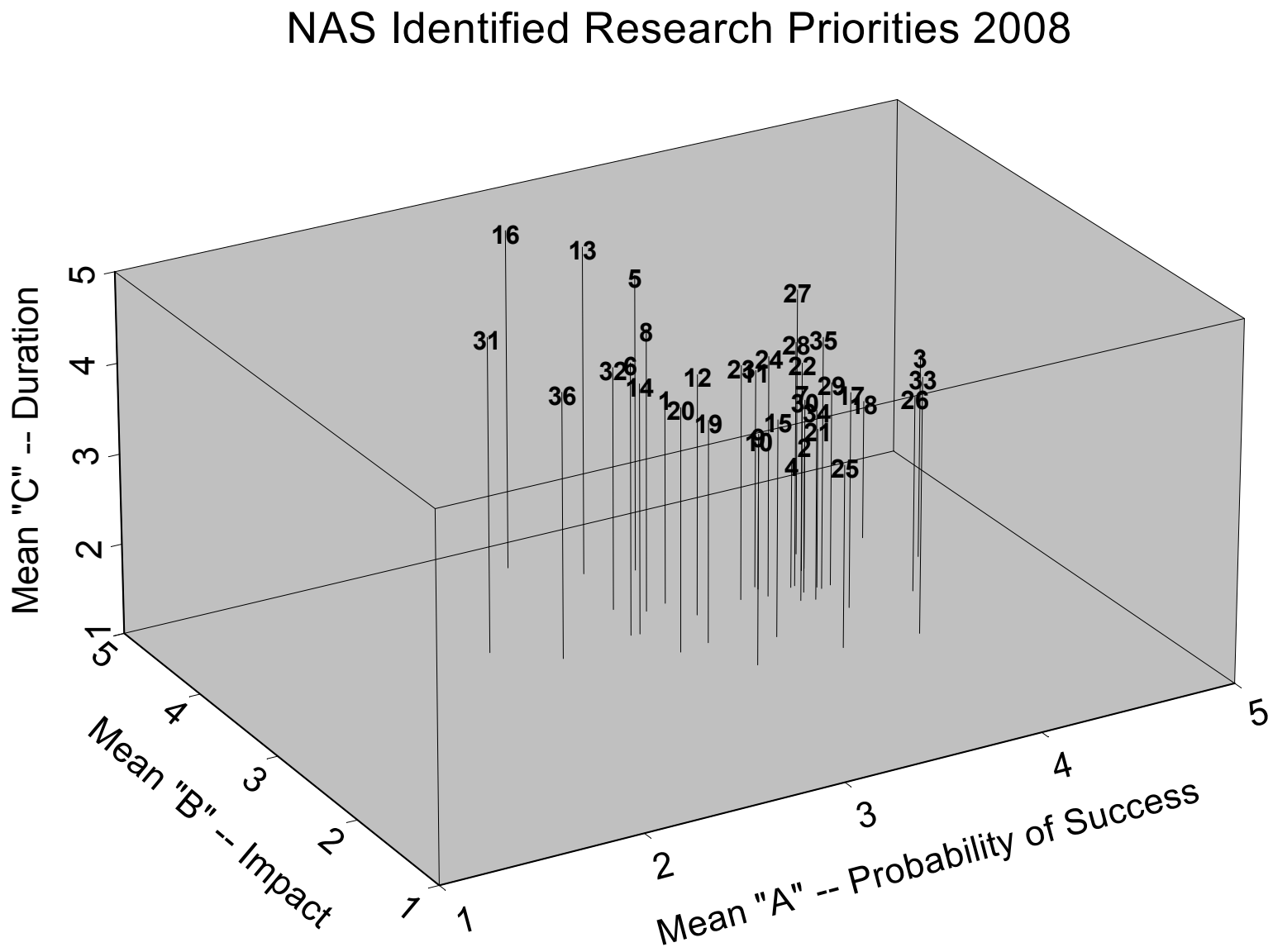

Figure 12. NAS Ranking of Impact vs. Success vs. Duration

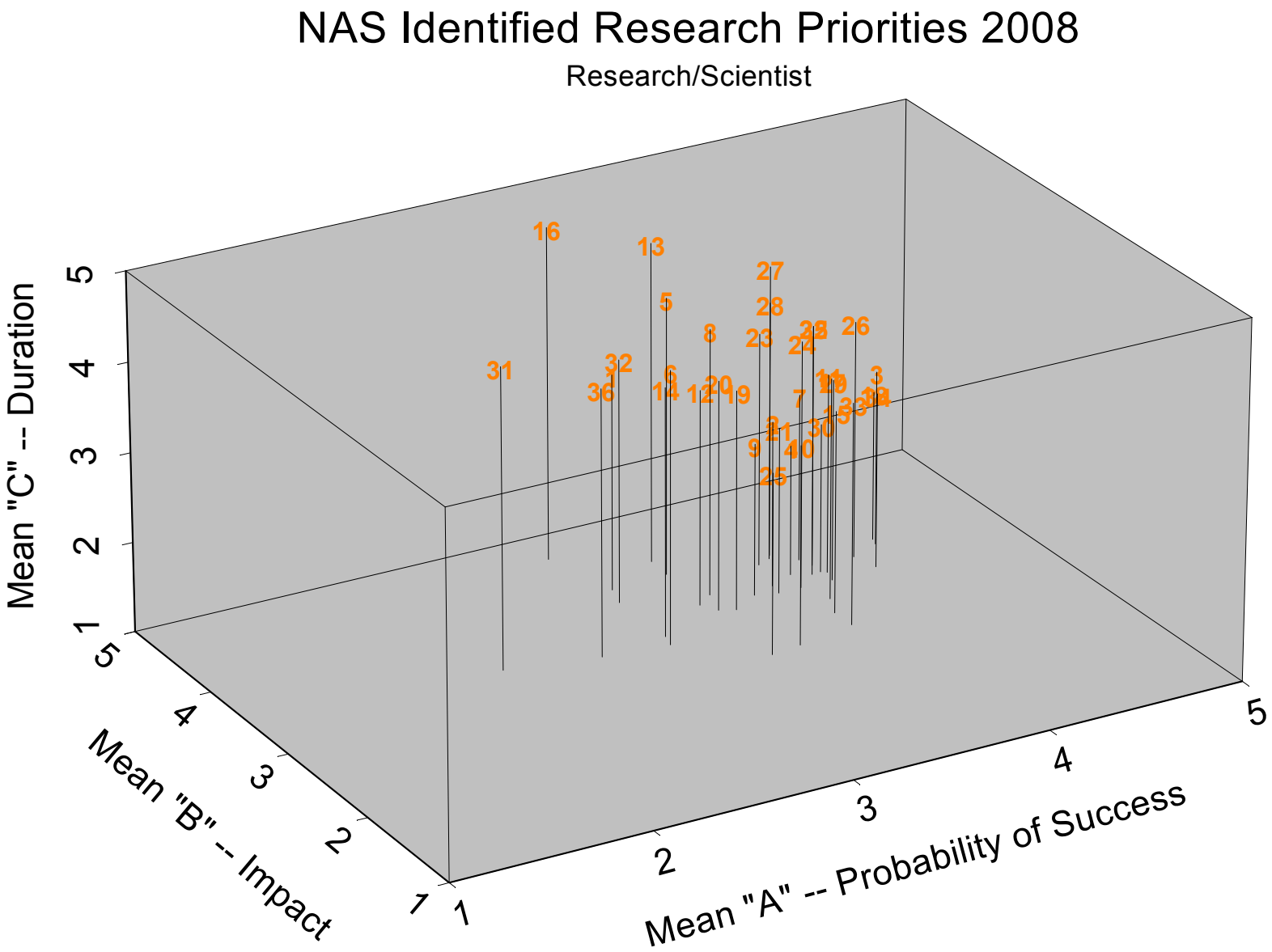

Figure 13. NAS Ranking of Impact vs. Success vs. Duration

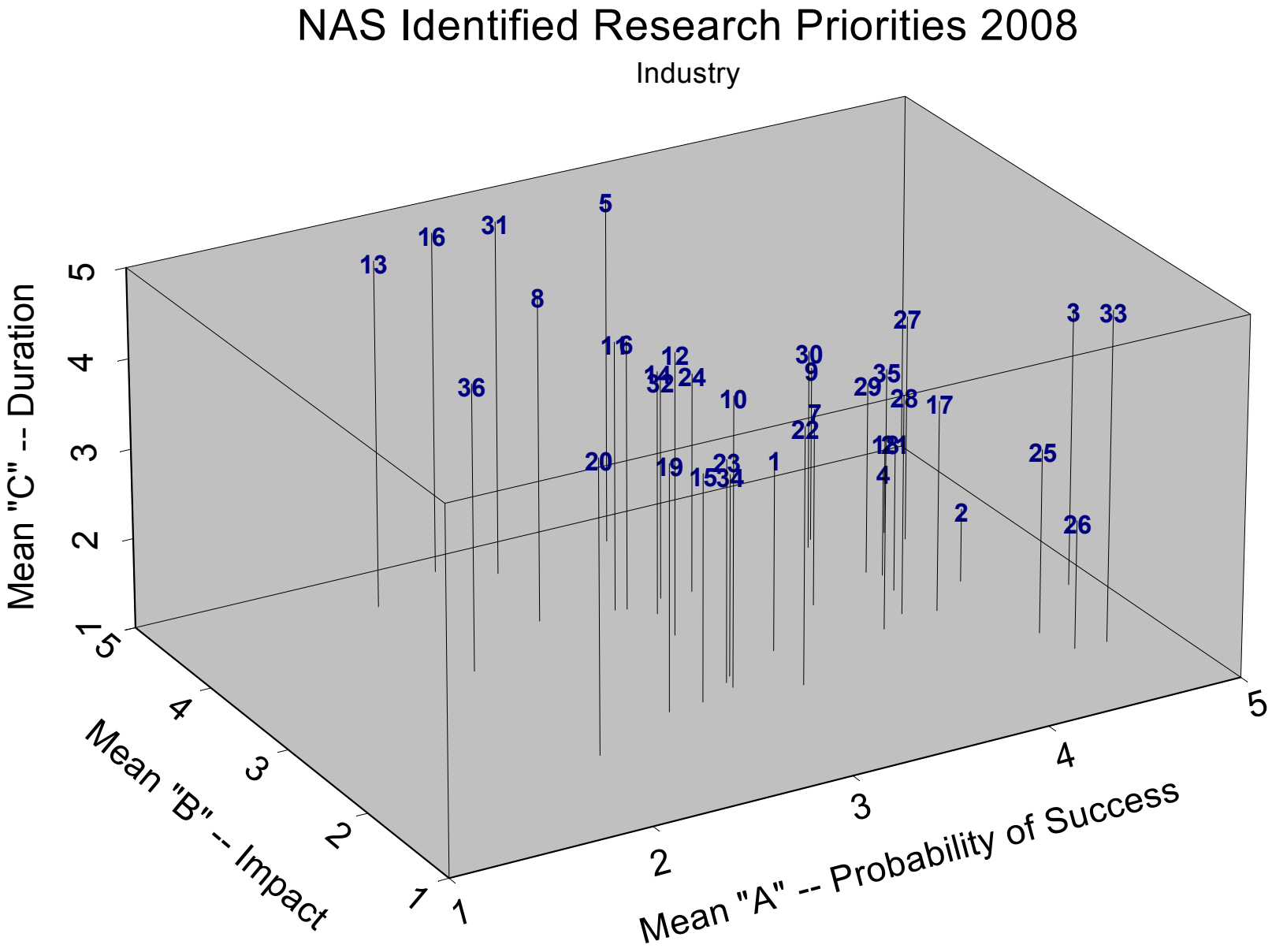

Figure 14. NAS Ranking of Impact vs. Success vs. Duration

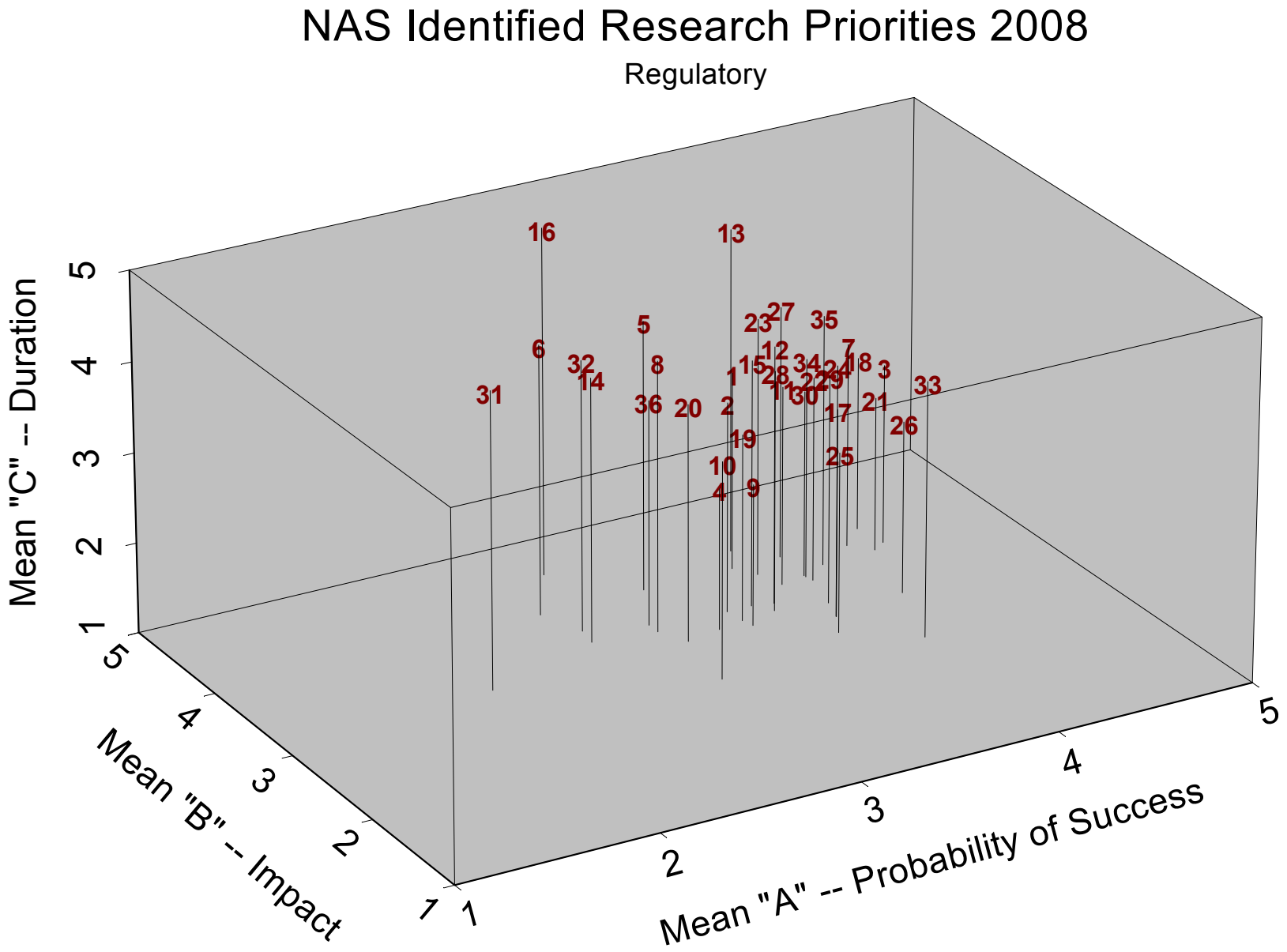

Figure 15. ARS Ranking of Impact vs. Success vs. Duration

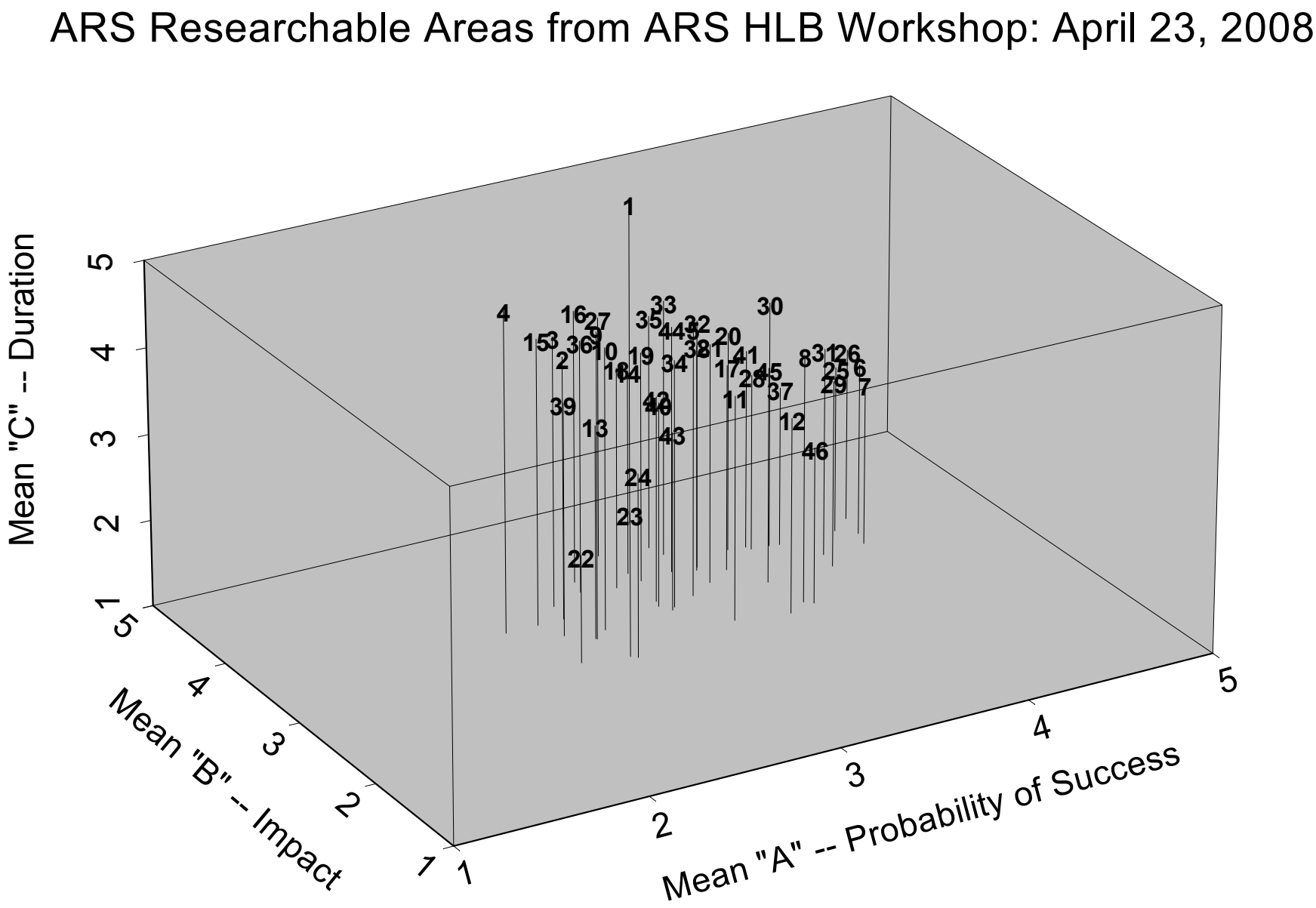

Figure 16. ARS Ranking of Impact vs. Success vs. Duration

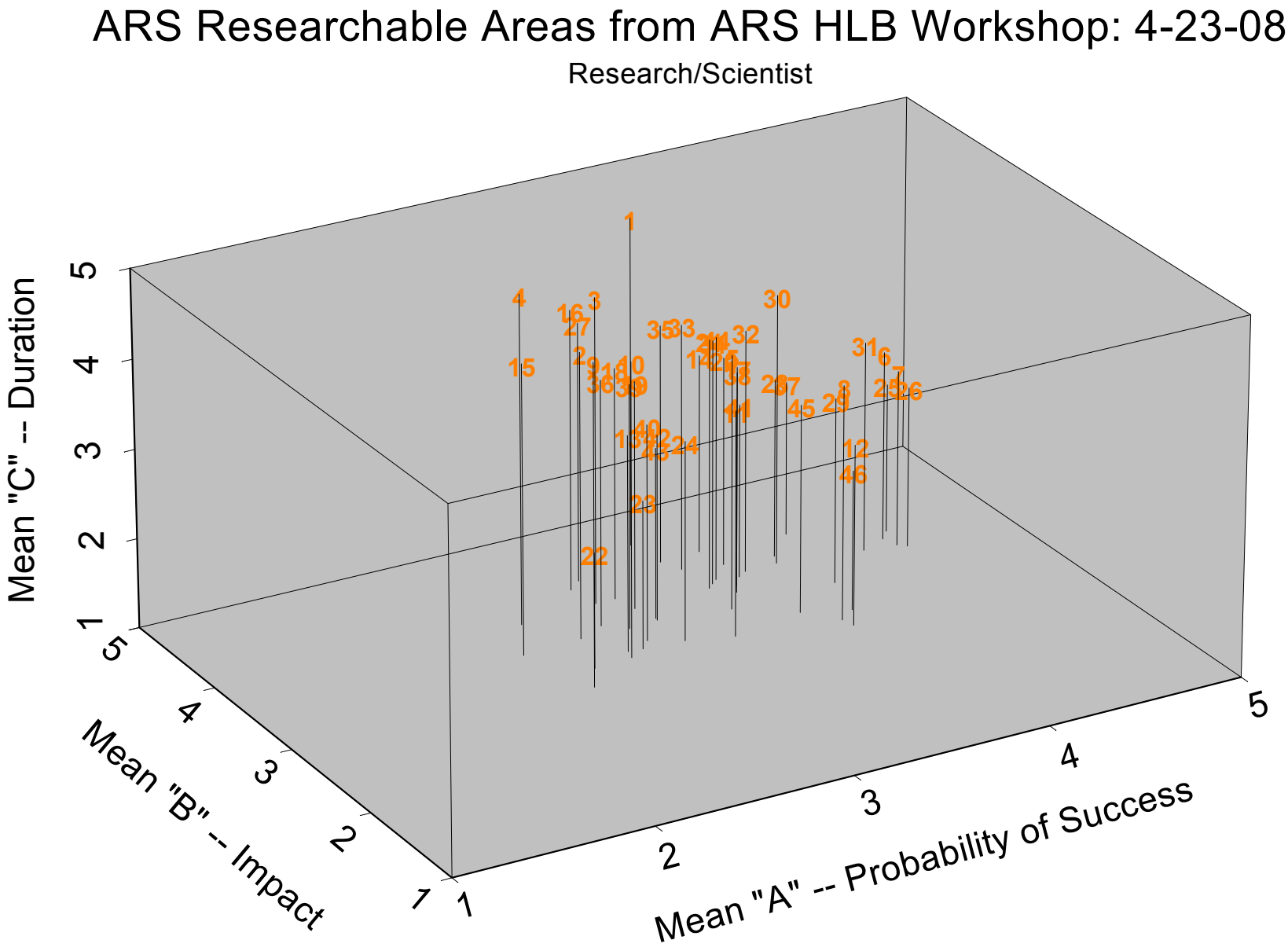

Figure 17. ARS Ranking of Impact vs. Success vs. Duration

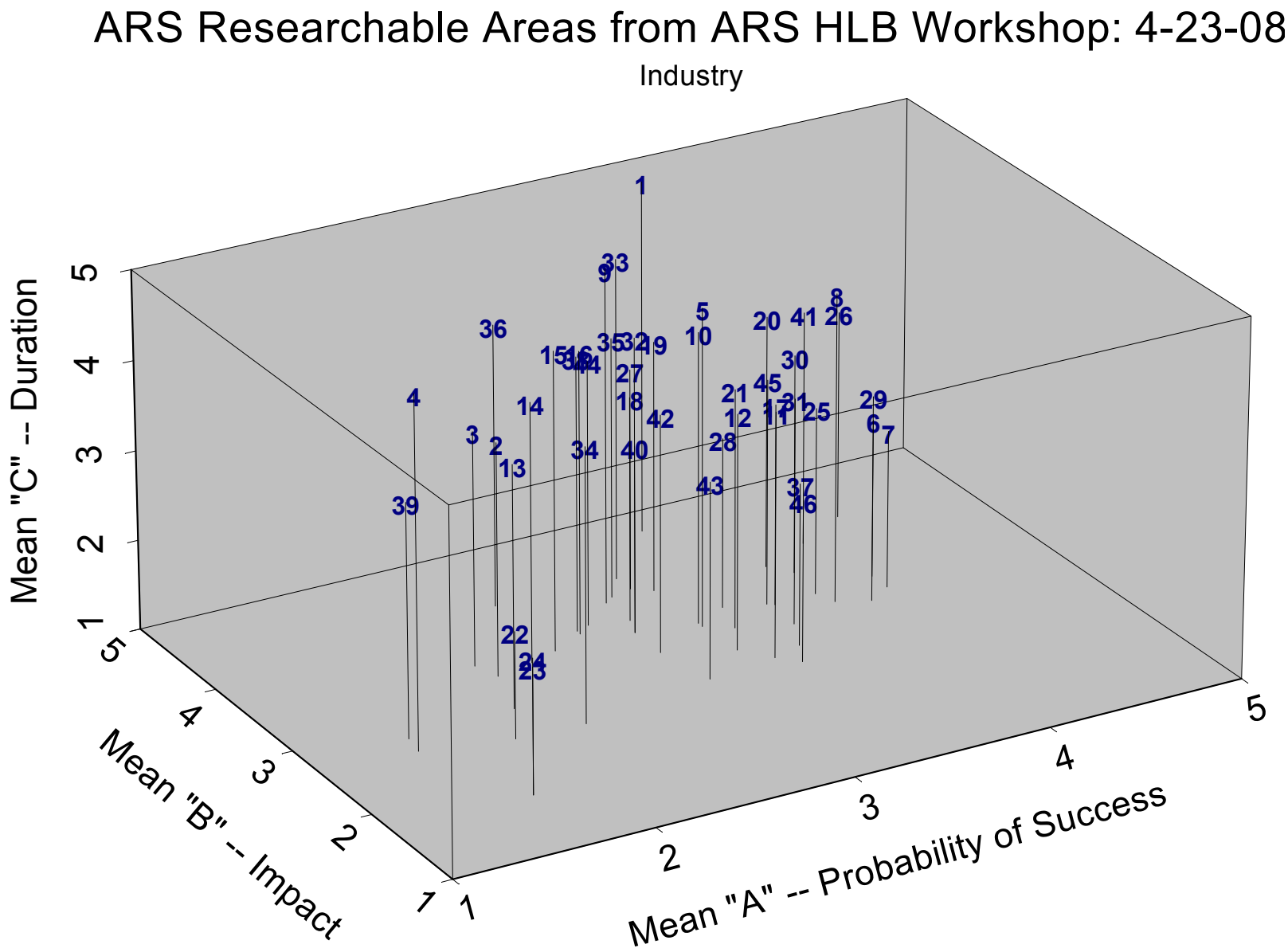

Figure 18. ARS Ranking of Impact vs. Success vs. Duration