Tree Improvement Branch

# Cone and Seed Improvement Program
# BCMoF Tree Seed Centre

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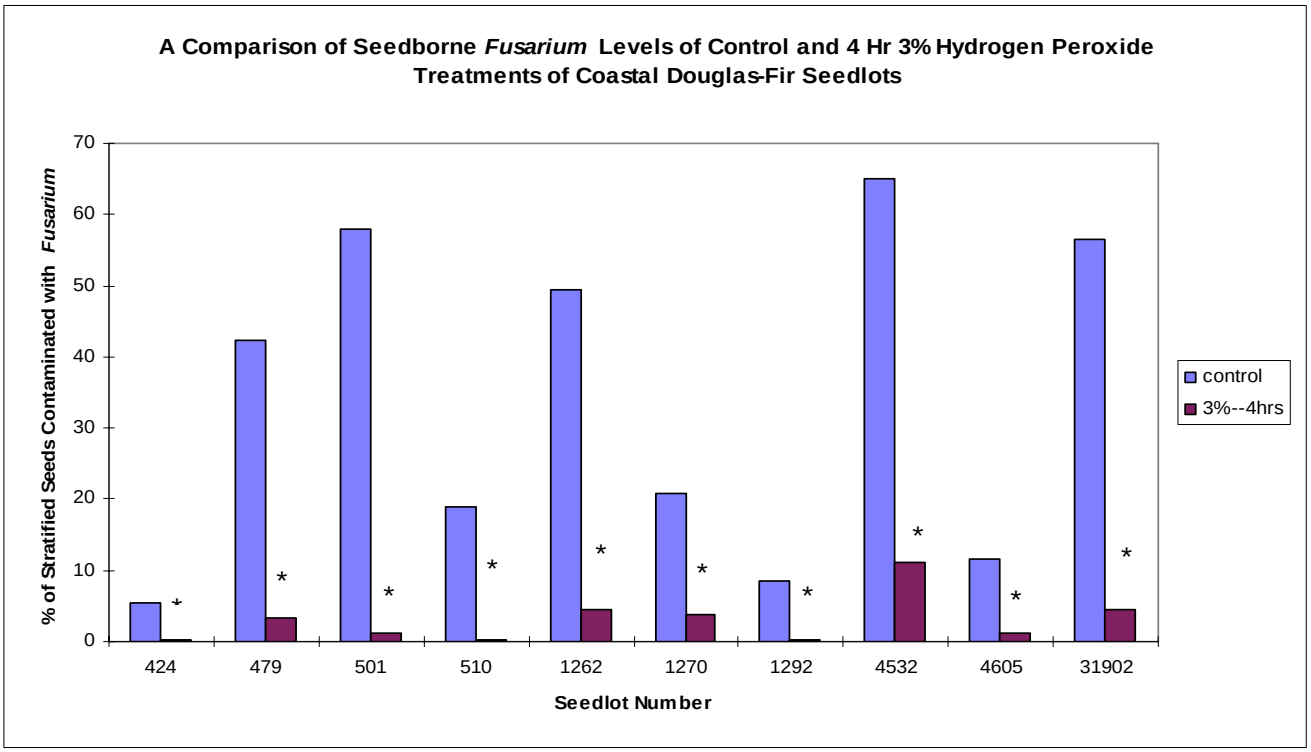
## SEED SANITATION METHOD TO REDUCE SEEDBORNE *FUSARIUM* LEVELS ON CONIFER SEED

### INTRODUCTION

The fungus *Fusarium* (Link) has been associated with damping-off and root rot of conifer seedlings in container nurseries. The seed has been shown to be a common source of this fungus. The use of running water to imbibe seed prior to stratification can play an important role in the reduction of seedborne *Fusarium* levels (Axelrood et al., 1995). However, for some seedlots further seed sanitation is required to reduce the risk of seedling losses at the nursery level. A pilot study of various seed sanitation methods (Axelrood and Trotter, unpublished data) showed that hydrogen peroxide was appropriate for seed sanitation since it reduced seedborne *Fusarium* levels without inhibiting germination. Following on from this work, a trial using hydrogen peroxide seed soaking treatments was performed on stratified coastal Douglas-fir, western larch, and *Abies lasiocarpa* (Bl) seedlots.

**Figure 1. A comparison of seedborne Fusarium levels of control and 4 Hr 3% Hydrogen Peroxide treatments of coastal Douglas-fir seedlots.**




* indicates that the sanitation treatment is significantly different from the control (p=0.01)

## METHODS

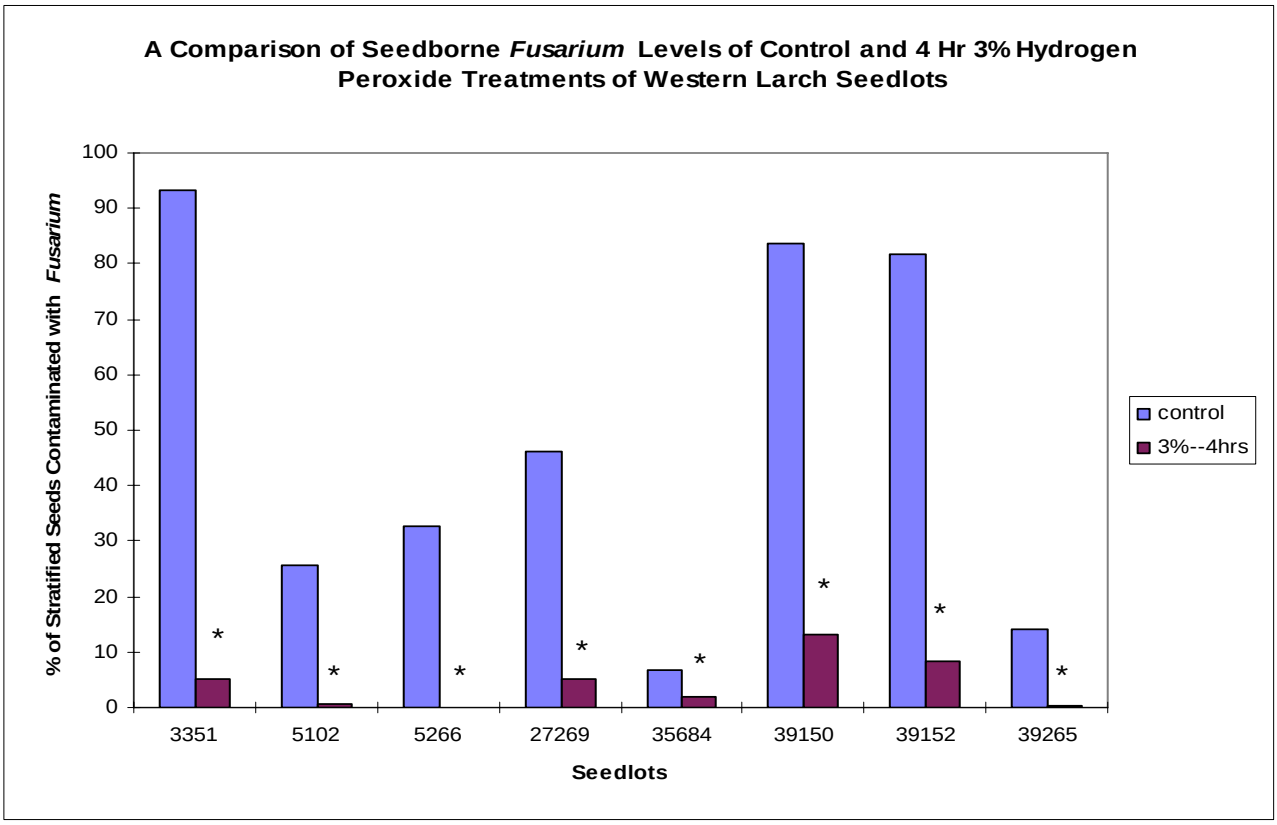
Seed from ten coastal Douglas-fir and *Abies lasiocarpa* and eight western larch seedlots were stratified operationally at the Tree Seed Centre. Seedlots with at least 0.2% *Fusarium* on dry seed (Tree Seed Fungal Assay Results, 1996) were chosen for the study. Following stratification, seed was assigned to one of the following treatments:

i) control (no further treatment)
ii) 1 hour soak in 3% hydrogen peroxide solution
iii) 4 hour soak in 3% hydrogen peroxide solution
iv) 16 hour soak in 3% hydrogen peroxide solution
v) 16 hour soak in 1% hydrogen peroxide solution.

After the hydrogen peroxide soaking, treatments (ii-v) were rinsed for 5 minutes under running tap water and surface-dried. Five hundred seeds were placed on Nash and Snyder medium and incubated under continuous fluorescent lighting for 10-14 days. The number of seeds contaminated with *Fusarium* were counted and the effectiveness of the treatment evaluated.

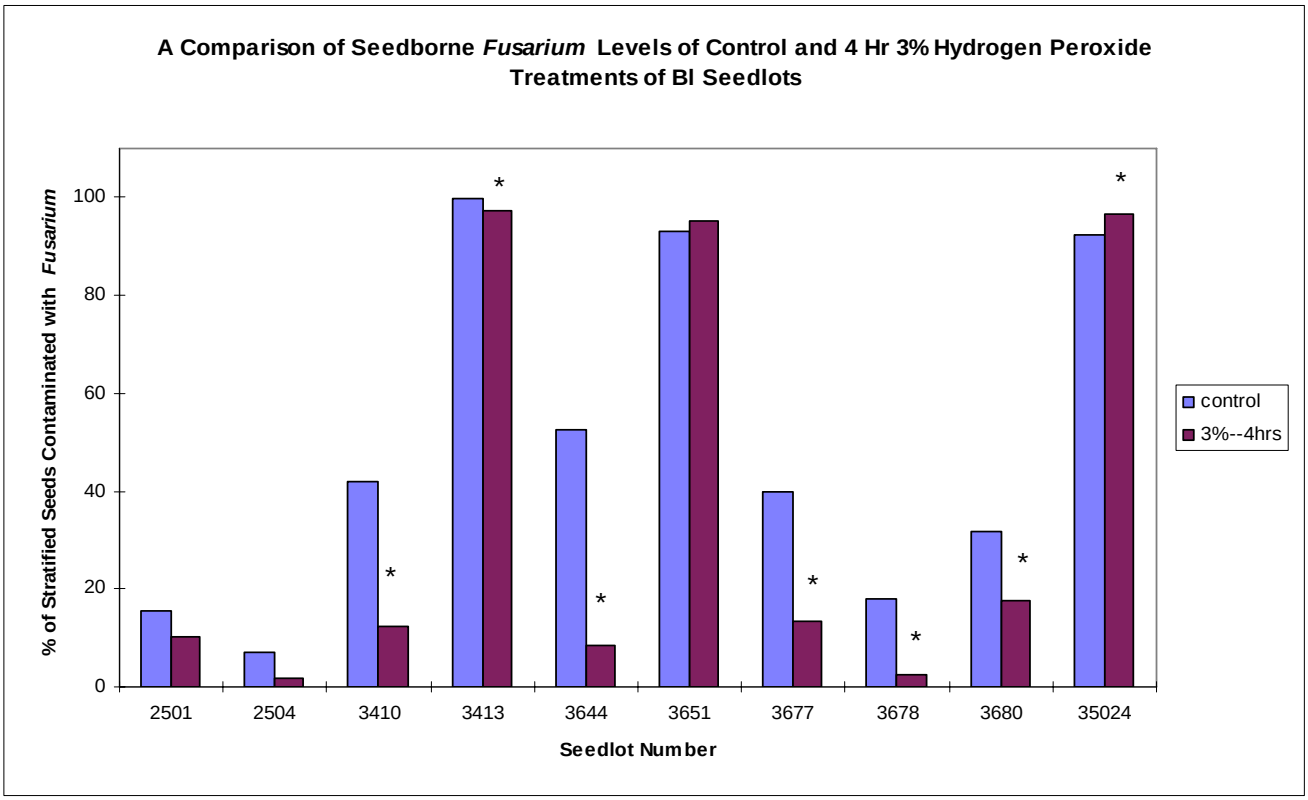
Field and laboratory germination tests of seedlot treatments were performed concurrently (Nursery Extension Services and the Tree Seed Centre).

**Figure 2. A comparison of seedborne Fusarium levels of control and 4 Hr 3% hydrogen Peroxide treatments of western larch seedlots.**




* indicates that the sanitation treatment is significantly different from the control (p=0.01)

**Figure 3. A Comparison of Seedborne *Fusarium* Levels of Control and 4 Hour 3% Hydrogen Peroxide treatment of *Abies lasiocarpa* Seedlots**




* indicates that the sanitation treatment is significantly different from the control (p=0.01)

## RESULTS

### Part A - Seed Assays

Hydrogen peroxide soak treatments were found to be very effective at reducing seedborne *Fusarium* levels. In general, the 3% 4 hour treatments provided the best reduction in *Fusarium* contamination. The 3% 1 hour treatment also provided good control whereas the 16 hour treatments were the most variable. The hydrogen peroxide seed sanitation treatments of *Abies* seedlots tended to be less effective and more variable than on the other two species tested. Figures 1-3 demonstrate that in most cases the 4 hour 3% hydrogen peroxide sanitation treatment significantly reduced seedborne *Fusarium* levels compared to the control treatment.

### Part B - Laboratory Germination

Results of total germination for all seedlots of the three conifer species are shown in Table 1. The treated seeds were placed in germination boxes in environmentally controlled incubators and germinants were counted 3 times per week for 3 weeks. For Douglas-fir (Fdc)and western larch (Lw), the incubators were maintained at $30^0$ C for 8 hours with lights and $20^0$ C for 16 hours with no lights. The *Abies lasiocarpa* (Bl) seed treatments were incubated under the same light setpoints but at $25^0$ C and $15^0$ C respectively. In general, the 3% hydrogen peroxide treatments at 1 and 4 hours showed little or no differences in germination compared to the controls in the Douglas-fir

and western larch seedlots. In contrast, only the 3% peroxide treatment for 1 hour was comparable to the control results for *Abies lasiocarpa* seedlots. In some of the *Abies* seedlots, germination was reduced with the 3% peroxide for 4 hours treatment. For all three conifer species, the two16 hour treatments increased the degree of germination variability across all seedlots and resulted in a greater percentage of seed with damaged seed coats.

**Part C - Field Germination**

Results of total germination under greenhouse conditions for all the tested seedlots are shown in Table 2. Stratified seed was single sown into cavities of 313a styrofoam blocks (198 cavities/block) loaded with a 1:3 vermiculite :peat media mix and then covered with a thin layer of forestry sand. The greenhouse environment was set to maintain a $20^0$ C soil temperature under an operational misting schedule. Overall, lower germination values were recorded across all seedlots and treatments compared to the laboratory results. For Fdc and Lw, 3% hydrogen peroxide for 1 or 4 hours resulted in germination values comparable to the controls. Sub-alpine fir germination was least affected by 3% hydrogen peroxide for 1 hour compared to the other treatments. Again, the 16 hour treatments resulted in greater variability coupled with reductions in total germination.

## CONCLUSIONS

Hydrogen peroxide seed sanitation treatments can be a very effective method of reducing seedborne *Fusarium* levels on contaminated seedlots. Consideration of both the pathology and field germination results leads to the conclusion that the 4 hour 3% hydrogen peroxide treatment of stratified seed is the best option for reducing *Fusarium* levels in Fdc and Lw. Although seed sanitation was the most effective nd consistent for the coastal Douglas-fir and western larch seedlots, considerable reductions in pathogen levels may still be obtained on *Abies lasiocarpa*. For sub-alpine fir, the 1 hour 3% treatment is the best alternative to the 4 hour treatment so as to reduce any impacts on germination. Neither of the 16 hour treatments are recommended since they resulted in seed coat breakage and poor germination.

## REFERENCES:

Axelrood, P.E., M. Neumann, D. Trotter, R. Radley, G. Shrimpton, and J. Dennis. 1995. Seedborne *Fusarium* on Douglas-fir: Pathogenicity and seed stratification method to decrease *Fusarium* contamination. New Forests 9: 35-51.

Neumann, M. 1997. Sanitation Methods for Conifer Seeds, Soaking Tanks and Screens to Control Seedborne *Fusarium*. Report submitted to BCMOF Nursery Extension Services, 49 pp.

**Table 1. Mean Total Percent Germination under Laboratory Conditions for Hydrogen Peroxide-treated Seedlots**

| Species | Seedlot | Control | SD | 3% for 1h | SD | 3% for 4h | SD | 3% for 16h | SD | 1% for 16h | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Fdc** | **424** | 86.0 | 3.3 | 85.0 | 4.5 | 85.3 | 2.8 | 84.8 | 2.2 | 84.8 | 1.5 |
| (n=4) | **479** | 85.0 | 4.4 | 85.5 | 0.6 | 87.8 | 2.9 | 88.5 | 4.0 | 86.5 | 2.4 |
| | **501** | 93.5 | 5.2 | 93.5 | 1.3 | 92.8 | 1.9 | 95.0 | 2.2 | 95.5 | 3.0 |
| | **510** | 95.0 | 2.4 | 92.3 | 3.0 | 93.3 | 1.0 | 95.0 | 1.8 | 94.0 | 2.2 |
| | **1262** | 83.3 | 3.9 | 80.5 | 4.4 | 81.0 | 7.3 | 77.3 | 4.3 | 73.0 | 2.2 |
| | **1270** | 86.5 | 0.6 | 81.3 | 5.7 | 80.3 | 2.5 | 81.5 | 1.7 | 82.8 | 2.8 |
| | **1292** | 78.3 | 5.3 | 75.0 | 9.7 | 77.3 | 3.0 | 75.3 | 5.0 | 72.3 | 3.3 |
| | **4532** | 85.0 | 4.8 | 84.8 | 3.4 | 87.0 | 2.3 | 78.0 | 3.5 | 78.3 | 3.9 |
| | **4605** | 86.0 | 4.3 | 77.8 | 4.1 | 88.3 | 1.7 | 86.0 | 3.5 | 88.5 | 2.5 |
| | **31902** | 95.5 | 2.4 | 93.8 | 1.9 | 94.5 | 2.1 | 94.8 | 2.9 | 94.8 | 3.3 |
| **Lw** | **3351** | 70.5 | 3.4 | 73.3 | 4.3 | 71.3 | 4.8 | 74.8 | 4.3 | 63.5 | 4.9 |
| (n=4) | **5102** | 83.5 | 3.3 | 86.0 | 4.5 | 89.3 | 2.5 | 91.0 | 3.4 | 88.0 | 1.4 |
| | **5266** | 93.5 | 2.6 | 95.5 | 2.6 | 93.5 | 2.4 | 94.5 | 3.1 | 93.8 | 1.7 |
| | **27269** | 88.0 | 2.6 | 84.0 | 4.2 | 85.0 | 0.8 | 87.5 | 2.1 | 87.3 | 5.6 |
| | **35684** | 80.0 | 5.0 | 82.5 | 5.0 | 82.5 | 5.1 | 82.0 | 4.1 | 77.5 | 5.1 |
| | **39150** | 66.5 | 2.1 | 67.0 | 3.8 | 70.0 | 4.7 | 71.8 | 6.6 | 66.5 | 1.3 |
| | **39152** | 45.0 | 3.9 | 46.0 | 3.7 | 49.3 | 4.6 | 50.5 | 3.8 | 41.3 | 3.3 |
| | **39265** | 84.3 | 5.5 | 87.5 | 2.5 | 85.8 | 4.3 | 87.5 | 4.8 | 86.8 | 3.9 |
| **Bl** | **2501** | 36.3 | 3.9 | 33.3 | 2.3 | 25.8 | 5.2 | 19.5 | 4.7 | 29.8 | 6.3 |
| (n=4) | **2504** | 53.0 | 3.7 | 44.0 | 2.7 | 44.3 | 4.3 | 54.3 | 5.1 | 37.0 | 7.9 |
| | **3410** | 36.0 | 2.7 | 38.0 | 5.8 | 34.5 | 4.1 | 19.3 | 8.1 | 21.3 | 6.2 |
| | **3413** | 29.5 | 4.4 | 26.0 | 6.3 | 24.0 | 6.3 | 11.0 | 4.1 | 23.8 | 7.0 |
| | **3644** | 45.5 | 5.9 | 46.3 | 3.9 | 48.8 | 5.1 | 35.8 | 4.7 | 30.5 | 2.5 |
| | **3651** | 3.3 | 0.5 | 1.8 | 1.0 | 2.0 | 1.4 | 1.5 | 1.3 | 3.0 | 2.2 |
| | **3677** | 56.3 | 10.0 | 56.5 | 3.9 | 54.8 | 4.9 | 48.5 | 7.4 | 35.0 | 6.4 |
| | **3678** | 22.0 | 3.2 | 22.3 | 3.0 | 30.0 | 2.9 | 30.0 | 4.4 | 19.5 | 4.5 |
| | **3680** | 17.8 | 5.7 | 19.0 | 2.7 | 17.8 | 5.0 | 16.3 | 5.7 | 8.3 | 3.3 |
| | **35024** | 3.5 | 1.7 | 3.3 | 2.1 | 3.0 | 3.2 | 2.0 | 0.8 | 2.0 | 1.4 |

Table 2. Mean Total Percent Germination under Greenhouse Conditions for Hydrogen Peroxide-treated Seedlots

| Species | Seedlot | Control | SD | 3% for 1h | SD | 3% for 4h | SD | 3% for 16h | SD | 1% for 16h | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Fdc** | **424** | 80.0 | 7.5 | 68.3 | 4.9 | 78.3 | 4.6 | 73.3 | 1.8 | 77.2 | 6.6 |
| (n=4) | **479** | 78.3 | 3.8 | 84.4 | 1.8 | 81.7 | 4.9 | 80.0 | 1.8 | 74.4 | 2.9 |
| | **501** | 87.2 | 8.0 | 92.2 | 4.3 | 96.1 | 2.1 | 92.8 | 4.6 | 90.6 | 6.4 |
| | **510** | 90.6 | 2.1 | 87.8 | 6.9 | 94.4 | 4.6 | 91.7 | 2.1 | 92.8 | 4.9 |
| | **1262** | 79.4 | 6.1 | 80.6 | 4.6 | 78.9 | 3.8 | 75.0 | 5.8 | 74.4 | 3.8 |
| | **1270** | 80.0 | 3.1 | 81.7 | 6.4 | 85.0 | 1.1 | 75.6 | 4.1 | 75.0 | 8.4 |
| | **1292** | 70.6 | 10.6 | 73.9 | 11.1 | 68.9 | 3.1 | 66.7 | 6.3 | 75.6 | 7.7 |
| | **4532** | 63.3 | 8.2 | 64.4 | 4.8 | 58.3 | 7.6 | 52.8 | 11.4 | 58.3 | 4.9 |
| | **4605** | 71.7 | 7.3 | 70.6 | 6.9 | 64.4 | 8.9 | 71.7 | 6.4 | 77.2 | 4.6 |
| | **31902** | 96.1 | 1.1 | 95.0 | 3.8 | 92.8 | 3.3 | 93.3 | 1.8 | 93.9 | 3.3 |
| **Lw** | **3351** | 45.6 | 6.7 | 40.6 | 8.4 | 45.6 | 7.8 | 33.3 | 6.5 | 42.8 | 7.3 |
| (n=4) | **5102** | 70.6 | 10.6 | 68.3 | 7.3 | 67.2 | 4.6 | 76.7 | 2.9 | 72.2 | 4.3 |
| | **5266** | 85.6 | 12.8 | 88.3 | 6.4 | 96.7 | 2.9 | 95.6 | 2.6 | 95.0 | 3.3 |
| | **27269** | 83.3 | 10.0 | 70.6 | 14.6 | 77.2 | 4.2 | 88.3 | 5.8 | 81.7 | 4.9 |
| | **35684** | 71.1 | 8.3 | 70.0 | 5.9 | 77.8 | 9.1 | 80.0 | 6.5 | 68.9 | 4.1 |
| | **39150** | 66.1 | 7.6 | 71.7 | 4.9 | 72.2 | 6.7 | 63.9 | 2.8 | 76.7 | 7.6 |
| | **39152** | 48.9 | 6.4 | 51.1 | 3.1 | 41.1 | 10.5 | 52.8 | 11.7 | 36.1 | 9.0 |
| | **39265** | 87.8 | 5.9 | 91.1 | 4.4 | 82.8 | 8.4 | 87.8 | 4.3 | 85.0 | 7.6 |
| **Bl** | **2501** | 29.4 | 5.8 | 28.3 | 10.3 | 18.3 | 6.1 | 22.8 | 3.8 | 26.1 | 6.9 |
| (n=4) | **2504** | 43.3 | 5.3 | 40.0 | 4.8 | 38.9 | 5.9 | 42.2 | 5.4 | 33.3 | 5.7 |
| | **3410** | 21.7 | 5.3 | 35.0 | 11.7 | 25.6 | 4.3 | 17.2 | 4.6 | 18.9 | 2.9 |
| | **3413** | 35.6 | 4.8 | 25.6 | 3.8 | 24.4 | 7.0 | 20.6 | 2.1 | 21.1 | 2.9 |
| | **3644** | 33.3 | 3.9 | 38.9 | 12.9 | 35.6 | 4.4 | 45.6 | 3.8 | 25.6 | 5.9 |
| | **3651** | 0.6 | 1.1 | 3.9 | 3.3 | 2.8 | 1.1 | 0.6 | 1.1 | 1.1 | 1.3 |
| | **3677** | 45.6 | 5.9 | 41.7 | 4.6 | 38.9 | 3.8 | 46.1 | 4.2 | 37.2 | 8.6 |
| | **3678** | 18.9 | 5.3 | 21.1 | 6.9 | 25.0 | 5.6 | 22.8 | 6.6 | 15.6 | 1.8 |
| | **3680** | 17.2 | 5.8 | 11.1 | 7.9 | 10.6 | 5.8 | 8.9 | 4.1 | 9.4 | 3.3 |
| | **35024** | 2.2 | 1.8 | 3.3 | 2.9 | 2.2 | 1.8 | 0.6 | 1.1 | 0.6 | 1.1 |

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